NRL Report 7067

Disinfection With Hypochlorite:

Application to Clothed Men, Construction Materials, and Electronic and Electrical Items

G. H. FIELDING, R. A. NEIHOF, AND W. H. ECHOLS

Protective Chemistry Branch
Chemistry Division

and

R. L. DIMMICK

Naval Biological Laboratory
Oakland, California

June 29, 1970

NAVAL RESEARCH LABORATORY
Washington, D.C.

CONTENTS

Abstract	ii
Problem Status	ii
Authorization	ii
INTRODUCTION	1
NOMENCLATURE NOTE	1
MATERIALS AND EQUIPMENT	1
Test Organism	1
Disinfectants	2
Extraction Solution	4
Subjects and Substrates, Experiments 1 and 2	5
Experiments 3 and 4	6
Experiments 5 and 6	6
Spray Devices	6
PROCEDURES	7
Experiment 1	8
Experiment 2	9
Experiment 3	9
Experiment 4	14
Experiment 5	14
Experiment 6	17
RESULTS AND BRIEF DISCUSSION	19
Experiment 1	19
Experiment 2	19
Experiment 3	22
Experiment 4	25
Experiment 5	28
Experiment 6	32
Corrosion-Sensitive Devices and Materials	36
Electrical and Electronic Devices	37
Difficultly Accessible Surfaces	37
Natural Materials	37
ASH, Regular and STB	37
GENERAL DISCUSSION	38
Man and Clothing	38
Materials of Construction	41
FUTURE WORK	41
SUMMARY AND CONCLUSIONS	42
RECOMMENDATIONS	43
ACKNOWLEDGMENTS	43
REFERENCES	44

ABSTRACT

The ASH/SLASH (Activated Solution of Hypochlorite/Self-Limiting Activated Solution of Hypochlorite) system, containing 0.2 percent hypochlorous acid, kills highly resistant bacterial spores on men and their clothing, on oily and greasy fabrics, on some two dozen different construction materials, and on some electrical and electronic items, without significant damage or degradation of the substrates.

PROBLEM STATUS

This is an interim report; work on the problem is continuing.

AUTHORIZATION

NRL Problem C08-22
Project S4801X2

Manuscript submitted February 2, 1970.

DISINFECTION WITH HYPOCHLORITE: APPLICATION TO CLOTHED MEN, CONSTRUCTION MATERIALS, AND ELECTRONIC AND ELECTRICAL ITEMS

INTRODUCTION

Small-scale laboratory studies at NRL during 1967-1968 resulted in development of new hypochlorite-based decontaminants: ASH and SLASH (1).* Both were highly effective when applied to *B. subtilis* spores on swatches of clothing fabrics. Both also gave indications of effectiveness and safety for disinfection of human subjects and construction materials. To check the NRL findings, and explore further the indicated potentialities of ASH and SLASH, studies at a specialized microbiological laboratory were necessary. The experienced and highly capable Naval Biological Laboratory (NBL) was ideal for this purpose. Through the Naval Facilities Engineering Command, cooperative arrangements were made for a series of six experiments, graded in scope and complexity, to be conducted during the winter and spring of 1968.

NOMENCLATURE NOTE

Although initial studies were directed toward BW defense problems, the findings are so promising for application to medical technology that the more general term "disinfection" has been substituted for "BW decontamination," used in prior reports.

MATERIALS AND EQUIPMENT

Test Organism

The spore of *B. anthracis* is highly resistant to both disinfectants and environmental effects; anthrax spores have been known to survive for 60 years under laboratory conditions (2); hence they ought to be an ideal test species. Such vitality naturally commends an organism for use in research on high-potency microbial disinfectants. However, in view of the virulence and high mortality rate of pulmonary anthrax infections, this organism is no longer widely used for disinfection studies.

Brazis et al. have shown that the spore of *B. subtilis* var *niger* is equal or slightly superior to the *B. anthracis* spore in resistance to hypochlorous acid and hypochlorites (3,4). Moreover, *B. subtilis* has been shown to be entirely innocuous. Consequently, in the NBL/NRL studies, as well as in studies at NRL (1) and in many other laboratories, *B. subtilis* has been the organism of choice.

Because of the broad-spectrum nonspecific effects of hypochlorite or chlorine solutions on all kinds and types of microorganisms (5), it is reasonable to assume that a decontamination system effective against *B. subtilis* spores will also be effective against anthrax spores and other less-resistant pathogenic organisms.

---

*ASH is an acronym for Activated Solution of Hypochlorite; SLASH is an acronym for Self-Limiting Activated Solution of Hypochlorite.

Disinfectants

Calcium hypochlorite has been used for a century or more as a microbial disinfectant in the form of bleaching powder, chloride of lime, etc. (6). The case of Semmelweis, in 1846, is especially well known. Often, however, hypochlorites have been, and are, used in an inefficient or almost inactive form. It is now generally understood that hypochlorites per se, in their naturally alkaline form, with the alkalinity frequently augmented to increase storage stability, are inherently low in bactericidal effect. In fact it is debatable whether strongly alkaline hypochlorites are bactericidal at all. It is only when hypochlorites are fortuitously or intentionally converted to hypochlorous acid that the well-known antibacterial action develops. Thus the very effective use of bleaching powder (in its normal, tropical, or supertropical grades) for water sterilization prior to the commercial availability of liquid chlorine depended on the conversion of hypochlorite to hypochlorous acid by the carbonic acid in water. A similar situation applies in the hypochlorite treatment of swimming pools, although overt acidification is at times resorted to in order to assure the desired conversion of hypochlorite ion to hypochlorous acid.

The magnitude of the effect of acidifying hypochlorites on bactericidal action has been recognized by chemists for well over 50 years, but it appears to be as yet incompletely recognized by life-science specialists. This intentional and controlled acidification is a basis of the ASH and SLASH disinfectants and is fundamental to the effective, economical use of hypochlorites in disinfection. It is sufficient to note that a small amount of a weak acid, such as sodium dihydrogen phosphate, reduces the pH to about 7 and converts the normally alkaline calcium hypochlorite solutions to hypochlorous acid and in so doing increases the bactericidal activity by a factor of several thousand. Accordingly, the use of ASH and SLASH, which was begun at NRL, was continued in the NBL/NRL effort.

Both ASH and SLASH contain hypochlorous acid derived from approximately 2000-ppm, or 0.2-percent, calcium hypochlorite. SLASH is differentiated by selected concentrations of sodium citrate, which reduce the hypochlorous acid at a controlled rate during and after completion of the antibacterial action. The sodium citrate can be regarded as a getter or scavenger for the excess hypochlorous acid. Corrosive effects of ASH are very low, compared with the usual bleach slurries containing over 10 percent (100,000 ppm) calcium hypochlorite, but the added sodium citrate in SLASH functions to reduce corrosion to a still lower level.

All ASH and SLASH chemicals for the NBL/NRL experiments were preweighed and packaged at NRL. The usual quantities were for 4 or 8 liters of solution. When the solutions are made with tapwater, more acid must sometimes be added to obtain a pH in the desired range of 6.8 to 7.0. Data for the NBL/NRL solutions are given in Table 1.

Figure 1 illustrates the type of plot used to determine the half-life of a SLASH solution from standard iodimetric titration data. The plotted data were obtained at NRL by mixing equal volumes of the two component solutions prepared from one of the sets of SLASH chemicals preweighed for the NBL/NRL experiments. As delivered by the two-tank apparatus to be described later, however, variations in half-life were common, due to unavoidable differences in flow rates of the components. The SLASH solutions used in Experiments 3 and 4 to be described should have shown titration plots and half-life identical with those of Fig. 1. In actuality the solution used in a preliminary trial and in Experiment 3 yielded the data shown in Fig. 2. The Experiment 4 solution, when checked after the experiment, gave a half-life of only 48 seconds. An inspection showed partial plugging of the filter screen in the hypochlorite line. This occurred some time after the beginning of disinfection; equality of the flow rates in the two solution lines was always established just before disinfection was started.

Table 1
Composition of Fully Mixed ASH and SLASH Solutions*

Component	Content (grams/liter)		
	ASH	ASH (STB)	SLASH
Calcium hypochlorite, 70% available chlorine	3	—	3
Supertropical bleach (STB), 30% available chlorine	—	7	—
Navy NBC detergent (decontamination compound)	5	5	5
Sodium dihydrogen phosphate monohydrate	4.5†	7†	—
Sodium citrate dihydrate	—	—	10‡
Citric acid monohydrate	—	—	2.1†

*SLASH is always made up in two solutions which separately contain the hypochlorite and citrate components at twice the concentration of the fully mixed SLASH. Equal volumes of the two solutions are mixed shortly before use. ASH is also conveniently made up in two solutions, hypochlorite and acid, for separate storage and mixing shortly before use.

†These amounts of acidic components are for solutions made up in distilled water to yield a pH of 6.8 to 7.0. When solutions are made up in tapwater, additional acid generally required has been about 2 g/l $NaH_2PO_4 \cdot H_2O$ for ASH, 4 g/l $NH_2PO_4 \cdot H_2O$ for ASH (STB), and 0.4 g/l of citric acid for SLASH.

‡This amount of citrate will yield a half-life in excess of 1 minute, but the half-life should be measured in each experiment with SLASH until reproducible mixing of the two solutions is assured.

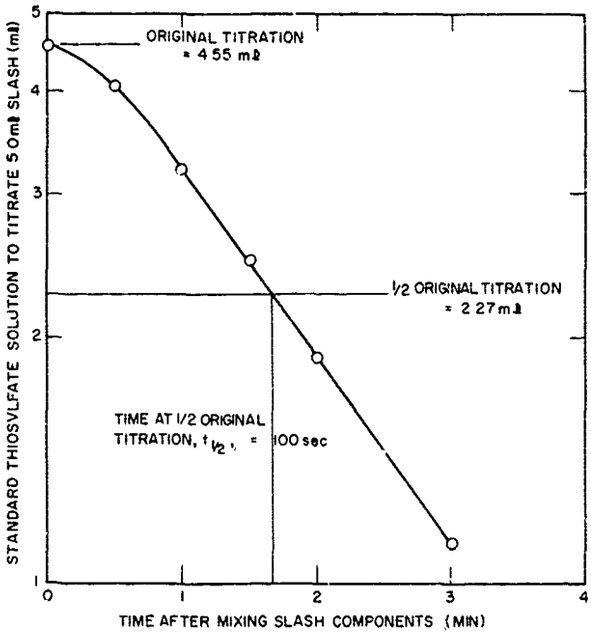


Fig. 1 - Titration plot used to check the half-life of SLASH for the NBL/NRL experiments (solution at 22°C)

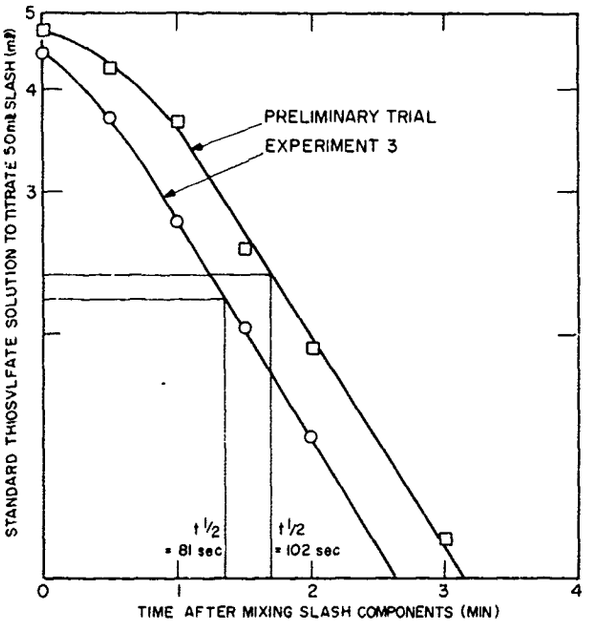


Fig. 2 - Titration plot showing the half-life of SLASH used in NBL/NRL Experiment 3

Extraction Solution

In Experiments 1 and 2 the solution used to extract *B. subtilis* spores from control and disinfected samples was prepared from autoclaved distilled water. Subsequently it was considered simpler to use common tapwater and sterilize it by 100 ppm of available chlorine as hypochlorous acid. This was conveniently supplied by 50 ml of ASH per liter of tapwater, plus the addition of about 2 ml of 1N NaH_2PO_4 solution to reduce the pH to 7.0. The data of Brazis et al. (3) extrapolated to 100 ppm suggest a time of 1.2 minutes for a 99.99-percent kill of resistant spores. An exposure time of 10 or 15 minutes was therefore considered to be ample. Then 4.7 g/l of sodium thiosulfate pentahydrate was added. The 0.7 g/l of sodium thiosulfate was required to neutralize the 0.1 g/l of calcium hypochlorite, leaving 4.0 g/l of sodium thiosulfate for the desired 0.4-percent solution.

To improve the wetting characteristics of the solution 0.5 g/l of Triton X-100* surfactant were added. This is well above the critical micelle concentration of approximately 0.01 percent needed to assure optimum detergency. A further component was added to the extraction solution for Experiments 4 through 6. This was 0.25 percent of the dispersing agent Daxad 11,† a sodium poly(alkyl naphthalene sulfonate) polyelectrolyte. The advantage of such a dispersant appears to be that it adsorbs onto the surfaces of spores, substrates, and containers, giving them all an abnormally high negative surface charge (7). The spores then repel each other electrically and are repelled by the substrate and the container walls; settling and redeposition of the spores is therefore minimized, and the precision and accuracy of viability assays are potentially improved.

*Rohm and Haas Co., Philadelphia, Pa.
†Dewey and Almy Chemical Co., Cambridge, Mass.

Marquis and Gerhardt (8) examined the effects of Daxad 11 and similar materials which were incorporated into bacterial growth media at concentrations as high as 2 percent. They stated: "The results of this investigation . . . indicate that these dispersing agents, in contrast to most wetting agents and detergents, are not injurious to biological material. This makes such dispersants suitable for use in microbiology, and their possible use has been indicated in plate-counting diluents, in direct counts, in the prevention of settling, and in the dispersion of pellicles. However, it also appeared that vigorous shaking may limit their utility and that their effects vary with different organisms and growth conditions. Therefore, it is necessary to test a given dispersant in a given situation."

The effects of both Daxad 11 and the Navy detergent* (containing 10 percent Triton X-100) had been examined briefly in connection with Experiment 11 of an NRL series not yet reported. A concentrated spore suspension containing about 5×10^5 organisms/ml was assayed by adding 1 ml of the suspension to 9 ml of water and plating the diluted suspension; the viability assay indicated 4.9×10^3 spores/ml. One ml of the concentrated suspension was then added to Daxad 11 and detergent solutions and allowed to stand for 4 or 22 hours before plating. The results are given in Table 2.

Table 2
Effects of Daxad 11 and a Detergent Containing
10 Percent Triton X-100 on *B. subtilis* Spores

Additive	Additive Concentration (%)	Time (hr)	Viability Assay (spores/ml)
Daxad	5	22	3.5×10^3
Daxad	5	4	4.0×10^3
Daxad	0.5	22	4.0×10^3
Daxad	0.5	4	3.8×10^3
NBC Detergent	5	22	3.7×10^3
NBC Detergent	5	4	3.5×10^3
NBC Detergent	0.5	22	4.2×10^3
NBC Detergent	0.5	4	3.3×10^3

It was concluded from the NRL data of Table 2 that the extraction solutions containing 0.05 percent Triton X-100 and 0.25 percent Daxad 11 used in the NBL/NRL studies would have no adverse effect on *B. subtilis* spores over a contact time of 4 hours, the maximum to be expected in the NBL/NRL experiments. However, NBL personnel believed that the additives in the NRL extraction solution may have been responsible for cases of abnormally slow colony development; in some cases three days were required for colonies to appear on zero-dilution plates. It is considered unwise, therefore, to use routinely the combination of Daxad 11 and the Navy detergent containing Triton X-100 in the extraction solution until more definitive data can be obtained on the limitations of use of these additives.

Subjects and Substrates, Experiments 1 and 2

The subjects used in Experiments 1 and 2 were two members of the staff of the NBC Defense School, Naval Schools Command, Treasure Island, San Francisco, where the

*Decontaminating compound FSN 6850-664-2008, MIL-C-7907 (Aer).

actual contamination and disinfection were done. The disinfection substrates were hybrid uniforms of blue nyco and green cotton sateen. Under these uniforms were worn impermeable rubber suits and boots. Gas masks were worn during the contamination step. This protection was adopted for the subjects, who were unfamiliar with the materials and procedures to be used, to eliminate any concern they might have had for their safety or well-being during the experiments. The uniforms were premarked with a wax pencil to delimit a 4-by-9-inch rectangular working area on each sleeve and trouser leg. Four disks of 5/8-inch diameter were punched from inside flaps of the uniforms and pinned to each rectangle. The disks were removed after contamination and combined in pairs as contamination controls.

Experiments 3 and 4

In Experiments 3 and 4, because the subject was an NRL member of the research team, some personal protective features were eliminated and a full-scale contamination and disinfection of both the subject and his clothing was carried out. The substrate was therefore the subject himself, plus his clothing. The latter included hybrid uniforms of nyco, sateen, and wool Melton cloth undress blues, together with underwear, gloves, nyco hoods, shoes and gas masks. All items were standard Navy issue except the underwear briefs. Swimming trunks were worn underneath the briefs to permit removal and assay of underwear without complete undressing.

Experiments 5 and 6

The decontamination substrates in Experiments 5 and 6 were 2-by-2-inch panels of a wide variety of outdoor and indoor construction materials, electronic and electrical equipment, a typewriter, some samples of earth and vegetation, and systematically oiled and greased nyco and wool fabrics.

Spray Devices

The spray devices involved in the experiments include the atomizer used for contamination in Experiments 1 through 4, the Collison nebulizer used for contamination in Experiments 5 and 6, the single-tank sprayer used for ASH, and the twin-tank sprayer used for SLASH.

As in most of the NRL experimental work, the clothing in Experiments 1 through 4 was contaminated with *B. subtilis* spores sprayed from a DeVilbiss No. 15 nasal atomizer. The air pressure was about 20 psi, as provided by the empty single-tank sprayer. The spray particle size given by this system has not been determined, but the spray is applied in sufficient quantity to result in considerable wetting of the substrate. It is assumed that contamination of clothing by a wet spray results in transport of spores deep into the fabric and yarn structure by capillary action.

In Experiments 5 and 6 a more realistic method of contamination was desired, so spores were disseminated in the form of a true aerosol. A classical device for this purpose is the Collison atomizer, which is capable of producing an aerosol, largely of single spores (9).

The single-tank sprayer* is a simple device consisting of a commercial 3-gallon garden sprayer modified for spraying ASH solutions. Its modifications consisted in coating the tank inside and out with a white epoxy enamel, replacing the original rubber hose with reinforced PVC tubing, and adding a pressure gage.

The twin-tank decontamination sprayer (Fig. 3a) is essentially two of the single-tank units rigidly mounted side by side, with a crossconnection to equalize the air pressure in each tank, and with the delivery hoses feeding into a valve-mixer-nozzle assembly (Fig. 3b). Two tanks are required to spray SLASH solutions. One tank contains calcium hypochlorite solution; the second tank contains sodium citrate, citric acid, and a detergent. When mixed, these solutions comprise SLASH, in which hypochlorous acid and citrate ion interact slowly and controllably to convert available hypochlorous acid to chloride ion.

Fig. 3a - Twin-tank decontamination sprayer

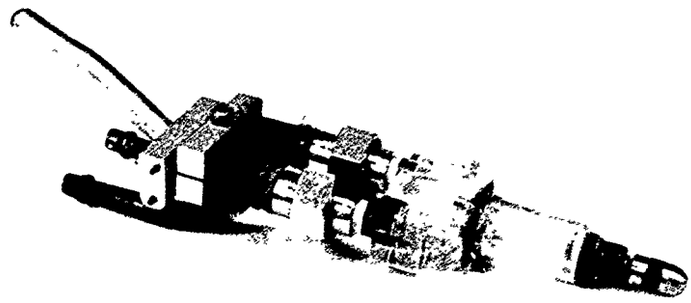


Fig. 3b - Valve-mixer-nozzle assembly

PROCEDURES

Certain procedures were essentially the same throughout all six NBL/NRL experiments. These were the extraction of spores from substrates and the viability assay for the numbers of viable spores extracted from substrates.

*"Yorktown" No. 21, D. B. Smith and Co., Utica, New York.

Spores were extracted from substrates at two different stages in the experiments: just after contamination and just after decontamination. The first extraction yielded the original degree of contamination of control specimens; the second extraction yielded the final degree of contamination of disinfected specimens. The ratio of the second degree of contamination to the first is the fraction of viable spores remaining after disinfection. In both cases the operation was identical. The swatch of clothing fabric or an entire garment, the piece of shoe or gas mask, or the panel of a construction material was mechanically agitated* in a test tube or hand-shaken in a plastic bag of extraction solution. This solution contained 4 g of sodium thiosulfate pentahydrate and 0.5 g of the nonionic surfactant Triton X-100, per liter. In Experiments 5 and 6 the solution also contained 2.5 g Daxad 11 per liter. The sampling of skin surface for spores followed a special procedure described in a following section.

The viability assay for spores was basically the drop-plate method of Miles and Misra (10), but special dropping pipets developed at NBL were used. In all cases 0.1 ml of the undiluted extraction solution, or decimal dilutions thereof, was plated in duplicate or triplicate on nutrient agar and incubated for 24 hours. Calculations of viable spores recovered per sample were straightforward: the number of colonies per plate was multiplied by the dilution factor of 10, 100, or 1000 to give the spores per 0.1 ml of the extraction solution and further multiplied by the factor required to give the number of spores in the entire volume of extraction solution, i.e., total spores per sample.

Experiment 1

Experiment 1 was planned as the first of two essentially identical experiments to be conducted on 24 and 25 January 1968. As will be seen, Experiment 1 was largely aborted because of equipment failure, but it was useful for familiarization.

All equipment was assembled at NBL. The screw-capped test tubes for samples were autoclaved, but the substrate clothing was not; the clothing had previously been thoroughly washed at NRL, however. The autoclaved extraction solution was loaded into the test tubes with a spring-loaded automatic syringe. The three ASH components were dissolved at NBL but were not mixed until shortly before use. The calcium hypochlorite and detergent were each dissolved separately in about 500 ml of water; one was then diluted to 1 liter, the other to 975 ml. The required sodium dihydrogen phosphate (NaH_2PO_4) was dissolved in 25 ml of water. All three components were later mixed at Treasure Island to give the required 2 liters of ASH. All chemicals had been preweighed and packaged at NRL.

At the NBC Defense School, Treasure Island, the plans and objectives were explained to the first day's subject: Wilburt L. Bowers FA B61 72 56. When the subject was dressed in the composite uniform over full-rubber protective clothing, each limb was covered with Saran Wrap plastic film, leaving a window over each of the four rectangles to be contaminated. The DeVilbiss atomizer was operated with water to check its performance. The atomizer operator was dressed in clear plastic (gloves, booties, and coveralls) to reduce accidental personal contamination and transfer of contamination to the laboratory. The atomizer was then loaded with a *B. subtilis* suspension containing 1 to 2×10^{10} spores per ml. The rectangles were sprayed from a distance of 6 to 8 inches, using four double passes over each rectangle.

Four control samples were then removed from each rectangle and combined in pairs in 10 ml of extraction solution to yield eight controls. After spraying a rectangle with

*"Vortex Jr." mixer, used for 30 sec per test tube.

ASH, the intent of the experiment was to punch out sample disks from the clothing at timed intervals to provide data on the degree of disinfection as a function of time. The mechanical punch failed after the left-leg samples were taken, and the first-day decontamination was ended at that point.

Air temperature, wind speed, and relative humidity were recorded. The ASH solution was analyzed spectrophotometrically via the starch-iodine complex before and after use, and its pH recorded.

Mr. Earl Beck, of the Naval Civil Engineering Laboratory, Port Hueneme, California, observed and assisted in the experimentation at the NBC School, as did Mr. Herbert Lacayo and Mr. Rolf Renner of the Naval Radiological Defense Laboratory (NRDL) and Mr. Fred Hauth of NBL. Photographic coverage was provided by Mr. John Schutz of NBL.

Experiment 2

Experiment 2, on 25 January, was carried out as planned. The preparations and procedures at NBL were identical with those for Experiment 1. At the NBC School the subject was Philman S. Skrove SA B62 64 93, who was dressed in a green cotton sateen shirt and nyco trousers over the full-rubber protective outfit. The plastic wrapping of arms and legs was eliminated as clumsy and impractical. As nearly as possible the contamination of the four marked rectangles was a duplication of that on the previous day. As a replacement procedure for punching out sample disks from the garments, squares about 1 inch on a side were cut out with scissors. To facilitate the timed removal of the samples following disinfection a number of parallel slits were cut 1 inch apart at the sample sites as soon as they were contaminated (Fig. 4a). After the ASH spray (Fig. 4b), and some 10 seconds prior to the end of a sample period, forceps were used to grasp the fabric between two of the slits, and two cross-cuts made with scissors to separate the square sample (Fig. 4c). At precisely the end of the sample period the square was dropped into 10 ml of extraction solution in a test tube held by one of the research team (Fig. 4d). The tube was then quickly capped and shaken. The sodium thiosulfate in the extraction solution served to neutralize the residual hypochlorous acid in the fabric sample and thus terminated the disinfectant action at the desired time. After each sample square was handled the forceps and scissors were dipped in ASH to eliminate any transfer of viable spores from one sample to another. The right leg and right arm were each sprayed once with ASH to an apparent condition of complete saturation. The left leg and left arm were sprayed in the same way, followed immediately by a similar additional spraying.

Environmental and analytical data were taken, as before. A thermometer placed in the sateen shirt pocket was read before and after the shirt was disinfected.

Experiment 3

Based on preliminary experiments at NRL on the disinfection of gas masks, shoes, and clothing fabrics, it was considered desirable to plan for a pair of experiments at NBL which would involve the one-step decontamination of a man, all his clothing, and gas mask and shoes. This was done, and the experiments were scheduled for 24 and 25 April 1968. As in Experiments 1 and 2 the decontaminations were performed at the NBC Defense School, Naval Schools Command, Treasure Island, alongside the dry-land training ship *Pandemonium*.

(a) Slitting of the subject's contaminated garment to facilitate removal of samples after the ASH spray

(b) Disinfection of the clothing with ASH spray

(c) Removal of samples from ASH-disinfected clothing

(d) Timed placement of a clothing sample in extraction solution

Fig. 1 - Procedures during Experiment 2, with the subject wearing a sateen shirt and nyco trousers

The clothing worn in Experiment 3 was the complete nyco work-combat uniform of shirt, trousers, and hood (Figs. 5a and 5b). The undershirt was Navy-issue cotton knit. Personal cotton knit briefs were worn over swimming trunks. Common knit cotton work gloves* were worn. Footwear was Navy-issue, high work shoes.†

Prior to contamination a window was cut in each sleeve to permit contamination of part of each forearm, and the edges of the fabric window were taped to the arm. Nyco disks, 5/8 inch in diameter, were fastened to each clothing item and to the skin with double-faced pressure-sensitive tape. Removal of these control disks after contamination and before disinfection allowed determination of the degree of contamination via viability assays.

The clothed subject was contaminated by spraying a *B. subtilis* suspension from the atomizer previously used (Fig. 5c). Pressurization was 20 psi of air, supplied by the single-tank decontamination apparatus. The subject was sprayed with two double vertical passes, head to foot, with a 45-degree turn made after each pair of double passes. As before, the atomizer operator was protected by plastic gloves, coveralls, and shoe covers, which were disinfected before removal (Fig. 5d). Following the contamination step the subject moved 50 feet upwind on a strip of plastic film. In this way contamination of the disinfection team and work area was minimized.

After the control disks were removed and placed in screw-capped test tubes for extraction (Figs. 5e and 5f), disinfection was carried out by spraying the clothed subject with SLASH (Figs. 5g and 5h). Following removal of the hood and gloves, the mask was removed and cut into three sections, and the various items were bagged (Fig. 5i). Sample rectangles were then cut, on premarked lines, from the shirt and trousers (Fig. 5j). All outer clothing items were removed, one at a time, and bagged for extraction and viability assay at NBL (Figs. 5k and 5l). Although the shirt was removed by slitting the sleeves and back, to avoid touching the forearm areas to be extracted, the entire garment was bagged as a single item. The mask and shoes were given special treatments involving a second overall SLASH spraying, and cutting of each item into several pieces (Fig. 5m). This was done to learn if there were tendencies for contamination to persist on any part. The mask was cut up with scissors into: headharness with attached buckles and tabs, the eyepiece with adjacent rubber, the diaphragm assembly with adjacent rubber, and the two canister mounts with adjacent rubber. The two canisters were removed and discarded. Decontamination of the canisters is believed feasible but requires a special study. Each shoe was cut up with a razor blade into: sole with adjacent leather, heel with adjacent leather, and upper.

When the outer clothing had been removed, the underwear was seen to be dry in some areas. Underwear items were therefore sprayed with SLASH (Fig. 5n) before removing them. The underwear was not assayed for residual spores in this experiment. The forearm skin areas were sampled for spores with extraction solution prior to removal of the T-shirt, to avoid cutting the shirt off the body (Figs. 5o and 5p).

The forearm skin areas were sampled with the regular extraction solution plus ultrasonic agitation. Two-ounce, narrow-mouth polyethylene bottles, with the bottoms cut off, were used as extraction solution containers. A bottle, with its cap screwed on, was inverted, and 40 ml of solution poured in the open bottom. The arm was brought over the inverted bottle, the open bottom was pressed firmly against the arm, and the arm and bottle were turned together until the bottle was upright. The bottle cap was now removed,

*Gloves, cloth, cotton, work, men's, Class I, MIL-G-1008E, DSA 1-8389, fabric 100% cotton.
†Shoes, service, men's, high, FSN 8430-144 MIL-S-17156.

(a) Clothed subject ready for contamination

(b) Clothed subject ready for contamination

(c) Contamination with an atomized spore suspension

(d) Disinfection of the atomizer operator

(e) Removal of a control sample from the contaminated clothing

(f) Placement of a control sample in extraction solution

(g) Disinfection of the clothed subject with SLASH

(h) Disinfection of the gas mask with SLASH

(i) Preparation of gas mask samples after disinfection

(j) Preparation of clothing samples after disinfection

(k) Removal of coat as a total sample after disinfection

(l) Removal of trousers as a total sample after disinfection

(m) Preparation of shoe samples after disinfection

(n) Disinfection of underwear with SLASH

(o) Extraction of forearm area after disinfection with SLASH

(p) Placing forearm extractant in capped test tube

Fig. 5 - Procedures during Experiment 3, with the subject wearing nyco clothing

and the horn of a 75-watt ultrasonic unit* was inserted to the maximum depth allowed by the length of the bottle, thus locating the horn tip about 1/4 inch from the skin. The ultrasonic generator was turned on, set at power level 4, and tuned to maximum output. After 30 seconds, the power unit was turned off, the bottle cap was replaced, the arm and bottle were reinverted, and the extraction solution was poured into a screw-capped test tube for viability assay.

With all clothing but the swimming trunks removed and bagged, the subject was sprayed once more with SLASH, and allowed to shower and dress. After the bagged clothing was returned to NBL the appropriate volumes of extraction solution were added and the bags were shaken by hand. Aliquots of the extraction solutions in test tubes and bags were then taken for viability assay.

Experiment 4

Experiment 4, on 25 April, was essentially a duplicate of that of the preceding day, except that the nyco shirt and trousers were replaced by the cotton sateen OD coat which is part of the standard U.S. fatigue uniform for land forces and the blue wool melton cloth trousers† which are a part of Navy undress blues. The footwear was the new chukka pattern "fleet" shoes.‡ All other clothing items were identical with those of Experiment 3. The subject was also the same. In this experiment the underwear also was assayed for residual spores after disinfection. Significant phases of the experiment are shown in Fig. 6.

A motion picture of Experiments 3 and 4 was prepared by NBL. This film is held by NRL Code 6140 and is available for viewing by interested persons.

Experiment 5

After the successful decontamination of personnel and clothing in the first four NBL/NRL experiments, attention was turned to other materials of particular interest to the Naval Facilities Engineering Command. Consideration was given to basing the final pair of experiments on the allover decontamination of weapons and motor vehicles. However, it was believed more appropriate and more useful to design extensive rather than intensive studies. A wide selection of exterior and interior building materials was therefore listed, and the Naval Civil Engineering Laboratory (NCEL) agreed to prepare suitable small samples.

The building-material samples, except ropes, were prepared as panels roughly 3 inches square by 1/2 inch thick. All were then coated by NCEL with an epoxy plastic on the back, sides, and edges of the sample face — leaving an exposed face nominally 3 inches square. At NBL each panel was additionally covered with paper taped in place, except for a face area about 2.5 inches square. The purpose of the coating and paper wrapping was to eliminate from consideration edge effects due to contamination of the sides and backs of samples, particularly of the controls. Thus each flat sample presented a uniform work area of natural surface, with the rest of the sample covered with a smooth nonporous epoxy surface on which any incidental contamination could be easily decontaminated. The NCEL samples included four specially made aluminum "crevice" fixtures, used to represent deep, narrow grooves, cracks, and crevices in machinery, buildings,

*Branson Sonifier Model S-75, Branson Instruments, Danbury, Conn.
†Trousers, men's, wool melton, blue, MIL-T-15495 (S&A).
‡Shoes, service, men's, chukka style, FSN 8430-904, MIL-S-21894.

(a) Contamination of the clothed subject

(b) Disinfection of the atomizer operator

(c) Removal of control sample disks from the contaminated clothing

(d) Disinfection of the hood

(e) Disinfection of the wool trousers

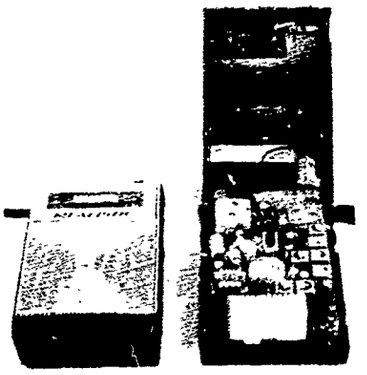
(f) Extraction from the forearm area after disinfection

Fig. 6 - Procedures during Experiment 4, with the subject wearing a sateen coat and wool trousers

etc. Each was made of two pieces of aluminum, 1/8 by 2 by 3 inches, one of which was slightly bent in a vise on a line midway between and parallel to the 3-inch edges. The two pieces were now bolted together on a line 1/2 inch from the 3-inch edges. The crevice between the two metal pieces was 3 inches long by 1 inch deep, tapering from about 1/16 inch at the face to zero at the root.

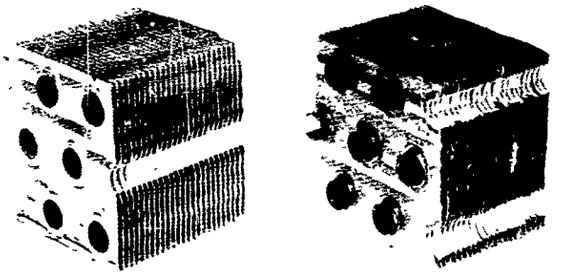
To the samples supplied by NCEL were added a typewriter, two AM/FM transistor radios (Fig. 7a), an electric drill (Fig. 7b), and clean and dirty finned-tube sections cut from an air-conditioning condenser (Fig. 7c). The latter were overall approximately 3-inch cubes, each comprising a stack of 25 thin aluminum sheets fitted over six copper tubes. The 48 surfaces 3 by 3 inches separated by 1/8 inch were relatively inaccessible to the SLASH spray and were thought to represent a substrate comparatively difficult to decontaminate. At NBL, samples of loamy and sandy soil and samples of hairy and smooth leaves were also assembled.

Contamination of all materials in Experiments 5 and 6 was by a distinctly different and probably more realistic procedure than in the earlier studies. All items were placed,



(a) Transistor radios after disinfection with SLASH

(b) Electric drill disinfected with SLASH



(c) Finned-tube sections disinfected with SLASH, precleaned (left) and dirty (right)

Fig. 7 - Some of the samples studied in Experiment 5

with special controls consisting of nyco disks and stainless steel squares, on the floor of a 1-cubic-meter aerosol chamber, which was then sealed. A *B. subtilis* spore suspension was sprayed into the chamber from a Collison atomizer. This device is known to produce an aerosol comprised largely of single spores when used with suspensions of appropriate concentration (9). After the aerosol had settled overnight, the special control samples were removed, together with a complete set of NCEL samples, one radio, the electric drill, and the typewriter. All were extracted for contamination controls, and the extracts were assayed for viability. A duplicate set of NCEL samples was then decontaminated, primarily with ASH, but some with SLASH, followed by extraction and viability assay.

Experiment 6

In Experiment 6 it was considered unnecessary to duplicate Experiment 5. Instead the decision was made to explore a number of new variables. One of these was the effectiveness of ASH made with supertropical bleach (STB) rather than with the calcium hypochlorite used in all of the previous studies on ASH. This variable was studied using 13 of the NCEL construction-material samples. The ones not used were those presumably the most easily disinfected and hence not a good test for the new ASH, those for which ASH would not be the disinfectant of choice, SLASH being preferred, and a few others. Of all the NCEL samples, only the acoustic tile remained soggy and unusable after drying overnight. Of the 13 pairs of construction material samples retained, both members of each pair were disinfected, one with regular ASH and one with ASH (STB) to afford a direct comparison of the two ASH types on each material. The extent of original contamination of these samples was calculated from that of eight cloth disks on the floor of the aerosol chamber.

Another group of substrates for disinfection was a series of fabrics in which certain special treatments were used with a view toward further exploring the potentialities and limitations of BW decontamination with hypochlorite. For baseline data, nyco, cotton sateen, and wool were contaminated as 3-inch squares. The controls for these fabrics were cut to 2-inch squares after contamination and were extracted, and the fluid was assayed for viability. The samples for disinfection were stretched on a trapezelike fixture (Fig. 8) and sprayed with decontaminant, and 2-inch squares were cut out for extraction and assay. The special treatments were:

1. "Quarpel" water-repellent treatment on a pair of nyco samples.

2. A pair of nyco and a pair of wool samples saturated with motor oil.

3. A pair of nyco and a pair of wool samples saturated with vegetable oil.

4. A pair of nyco and a pair of wool samples saturated with petroleum grease.

5. A pair of nyco samples in which an effort was made to drive the aerosol-deposited spores into the finest interstices of the fabric by placing a pair of 3-inch contaminated squares face-to-face in a small polyethylene bag, and slapping the bag hard against the edge of the laboratory bench 100 times.

6. A contaminated nyco square which was enclosed in a one-layer envelope of clean nyco before decontaminating.

7. A contaminated nyco square which was enclosed in a two-layer envelope of clean nyco before decontaminating.

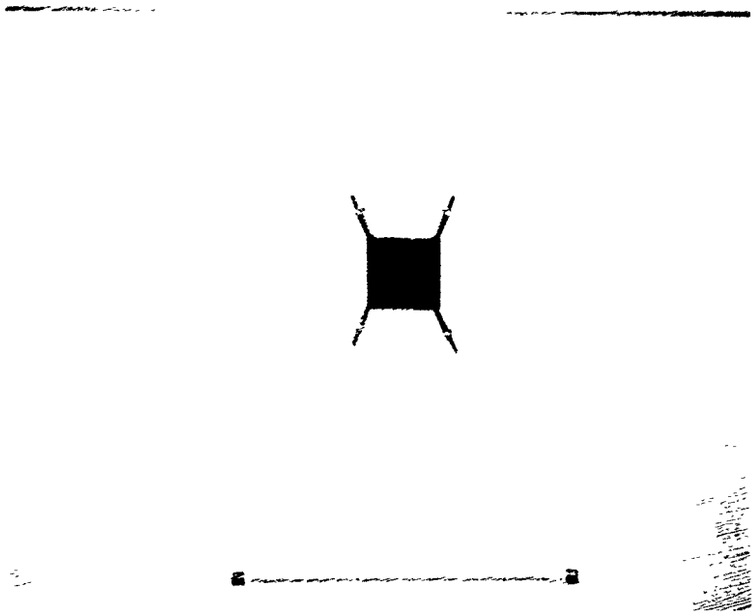


Fig. 8 - Trapeze support of 3-inch nyco squares for disinfection

The oily cloth samples were prepared by the following procedure. The 3-by-3-inch squares were each weighed and marked, soaked in the oil, and well blotted. As a final step, each square was placed between paper towels and 1/4-inch glass plates and weighted with a lead brick. The towels were changed several times over a period of 2 hours. The greasy squares were prepared in a similar way, with the difference that the grease was worked into both sides of the fabric with a narrow spatula, and then scraped off. After being weighed the samples were wrapped in aluminum foil. The data are given in Table 3.

The third group of special decontamination substrates included the two AM/FM radios, the electric drill, the typewriter, the dirty and clean 3-inch cubical sections of finned tubing (Fig. 7b) cut from an air conditioner condenser, smooth leaves (magnolia grandiflora), hairy leaves (probably a thistle or similar to a thistle), sandy soil, and loamy soil.

Finally, three samples of contaminated nyco were disinfected with "0.25-percent SLASH." The SLASH component solutions were the usual citrate solution unchanged, and the usual hypochlorite solution diluted with water to 25 percent of normal. The effect of this modification was to reduce the initial hypochlorite concentration in the nyco by a factor of four, and to lengthen the half-life considerably. However, all samples in Experiments 5 and 6 were placed in the extraction solution about 1 minute after spraying. In all cases, therefore, disinfectant action was limited to approximately 1 minute.

Table 3
Preparation of Oily/Greasy Cloth Samples for Experiment 6

Cloth	Dry Weight (g)	Additive	Treated Weight (g)	Additive Weight (g)	Pickup of Additive (%)
Nyco	1.716	Engine oil*	2.473	0.757	44.1
Nyco	1.639	Engine oil*	2.347	0.708	43.2
Nyco	1.860	Vegetable oil†	2.568	0.708	38.1
Nyco	1.677	Vegetable oil†	2.354	0.677	40.4
Nyco	1.818	Lubricating grease‡	2.982	0.164	64.0
Nyco	1.847	Lubricating grease‡	3.050	1.201	65.0
Wool flannel	1.488	Engine oil	2.225	0.737	49.5
Wool flannel	1.449	Engine oil	2.177	0.728	50.2
Wool flannel	1.464	Vegetable oil	2.270	0.806	55.1
Wool flannel	1.443	Vegetable oil	2.124	0.681	47.2
Wool flannel	1.463	Lubricating grease	2.681	1.218	83.3
Wool flannel	1.450	Lubricating grease	2.677	1.227	84.6

*Mobiloil Special SAE 10W-20W-30.
†Wesson Oil (cottonseed oil).
‡Mobil wheel-bearing grease.

RESULTS AND BRIEF DISCUSSION

Experiment 1

The results of Experiment 1 are given in Table 4. The degree of contamination attained averaged 14×10^7 spores per 5/8-inch (1.6-cm) disk, or 7×10^7 spores per square centimeter. The disinfection effectiveness was less than expected but was satisfactory at 99.8 percent after 40 seconds exposure to ASH. The ASH solution in the sprayer was found to contain 2250 ppm available chlorine before the subject was decontaminated and 1800 ppm after. The ASH pH was 7.2; at this pH the total hypochlorite is 64 percent in the form of hypochlorous acid. The somewhat high pH can be attributed to the use of tapwater in making up the ASH. The amount of the acidifying agent NaH_2PO_4 prepackaged at NRL was based on the amount determined for the use of distilled water.

The air temperature at the decontamination site was 57°F, the wind speed was 6 to 8 mph, and the relative humidity was about 50 percent. No measurement of the surface temperature of the clothing was made.

Experiment 2

Table 5 presents the results of Experiment 2. Two noteworthy facts appear: at three of the four disinfection sites the disinfection was extremely effective, but at the fourth site, the sleeve on the right arm, the effectiveness was variable and fell as low as 98.2 percent. The right arm and the right leg of the uniform were sprayed only once with ASH, but the left limbs were sprayed twice. Thus, the results suggest that a double ASH spray of a uniform is more likely to achieve complete decontamination than is a single spray; a single spray, however, is capable of complete disinfection. It is recognized that the incomplete disinfection of the single-sprayed right arm. in comparison with the single-sprayed right leg, could equally well be accounted for by some inhibitory effect of the cotton sateen shirt not shown by the nyco trousers. This latter explanation has not

Table 4
Experiment 1: Viability Assays and Disinfection Effectiveness

Sample	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution					Viable Spores Recovered Per Sample	Disinfection (%)
	Undiluted	0.1X	0.01X	0.001X	0.0001X		
Left Leg							
Control	—	—	—	—	66,87,72	7.5×10^7	—
Control	—	—	—	—	ca 2000	ca 2×10^9*	—
Av control	—	—	—	—	—	7.5×10^7	—
19-sec decon	—	—	209,184, 182	15,18,21	—	1.9×10^6	97.5
30-sec decon	—	—	44,40,60	5,4,4	0,1,0	4.8×10^5	99.4
40-sec decon	—	125,119, 114	—	—	—	1.2×10^5	99.8
Right Leg							
Control	—	—	—	—	58,81,69	6.9×10^7	—
Control	—	—	—	—	86,77,80	8.1×10^7	—
Av control	—	—	—	—	—	7.5×10^7	—
Left Arm							
Control	—	—	—	—	266,294, 285	2.8×10^8	—
Control	—	—	—	—	336,253, 311	3.0×10^8	—
Av control	—	—	—	—	—	2.9×10^8	—
Right Arm							
Control	—	—	—	—	—	—	—
Control	—	—	—	—	—	—	—
Av control	—	—	—	—	—	—	—

*Not included in the average.

been adopted, because the pertinent physical properties of both fabrics appear to be the same; i.e., they are of equal weight per unit area, both are tightly woven, and both are rapidly wetted by ASH. If the chemical differences in the fabrics are significant in disinfection susceptibility, it would be expected that the nylon-containing nyco would react more readily with hypochlorous acid and so would be less susceptible to disinfection. Since it was the sateen garment which was incompletely disinfected with one spray, the former explanation is preferred.

Table 5
Experiment 2: Viability Assays and Disinfection Effectiveness

Sample	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution					Viable Spores Recovered Per Sample	Disinfection (%)
	Undiluted	0.1X	0.01X	0.001X	0.0001X		
Right Leg							
Control	TNTC*	TNTC	TNTC	TNTC	26,40,26	3.1×10^7	
Control	TNTC	TNTC	TNTC	115,122, 140	15,12,23	1.3×10^7	
Av control						2.2×10^7	
25 sec	1,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.999+
52 sec	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.999+
80 sec	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.999+
110 sec	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.999+
140 sec	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.999+
165 sec	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.999+
180 sec	1,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.999+
Left Leg							
Control	TNTC	TNTC	TNTC	104,96, 80	13,12,11	9.3×10^6	
Control	TNTC	TNTC	TNTC	27,26,24	2,1,0	2.6×10^6	
Av control						6.0×10^6	
30 sec	2,0,0	0,0,0	0,0,0	0,0,0	0,0,0	1×10^2	99.998
50 sec	1,1,1	1,2,0	0,0,0	0,0,0	0,0,0	1×10^2	99.998
80 sec	0,0,0	0,0,0	0,0,0	1,0,0	0,0,0	0	99.998+
110 sec	6,8,6	1,0,1	0,0,0	0,0,0	0,0,0	7×10^2	99.99
150 sec	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.998+
180 sec	0,1,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.998+
Right Sleeve							
Control	TNTC	TNTC	TNTC	TNTC	10,20,14	1.5×10^7	
Control	TNTC	TNTC	TNTC	TNTC	8,3,9	0.7×10^7	
Av control						1.1×10^7	
30-sec decon	2,0,0	0	0	0	0	1×10^2	99.999+
55-sec decon	300,276, 299	15,40,22	252,110, 53	103,90, 102	ca 100	2.9×10^4	99.83
80-sec decon	ca 100	7,8,13	3,0,1	0,0,0	0,0,0	9×10^3	99.92
110-sec decon	TNTC	80,340, 153	3,110,75	1,0,0	0,0,0	2×10^5	98.2
150-sec decon	TNTC	TNTC	17,13,20	50,55,50	TNTC	2×10^5	98.2
180-sec decon	ca 40	40,40,50	ca 200	16,40,50	60,80, 100	4×10^4	99.6
Left Sleeve							
Control	TNTC	TNTC	TNTC	68,71,67	9,8,4	6.9×10^6	
Control	TNTC	TNTC	TNTC	66,93,62	124,164, 134	7.4×10^6	
Av control						7.1×10^6	
30 sec	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.999+
50 sec	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.999+
80 sec	0,0,0	0,0,0	0,0,0	1,0,0	4,0,0	0	99.999+
110 sec	0,0,0	0,0,0	0,0,0	1,0,0	0,0,0	0	99.999+
140 sec	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0	99.999+
160 sec	0,0,0	0,0,0	0,0,0	2,0,0	0,0,0	0	99.999+
180 sec	3,1,1	1,0,0	0,0,0	0,0,0	0,0,0	2×10^2	99.9972

*Too numerous to count.

Experiment 3

The results of Experiment 3 are given in Tables 6, 7, and 8. Both the usual drop-plate method and the membrane filter method were used for viability assay, because the disinfection efficiencies in this pioneering experiment were considered sufficiently unpredictable that it would be unwise to depend entirely on one analytical technique. Both approaches yielded good results, and in general the results are in excellent agreement. The major achievement, however, was the disinfection of a man and all his outer clothing in a single integrated treatment.

The air temperature varied from 14°C to 17°C (57°F to 65°F) during the experiment, the relative humidity was 43 percent, and the wind speed was 0 to 2 mph.

The concentration of the spore suspension was 1.6×10^{10} per ml. The volume sprayed was 10.5 ml, equivalent to 1.7×10^{11} spores. The total of the spores deposited on the clothing, as calculated from controls and the estimated areas was 1.07 10^{11}. That is, 63 percent of the spores sprayed were deposited. This is a reasonable figure in view of the lack of data on the true areas of the clothing items, of the efficiency of deposition of the atomizer spray, and of the degree to which the contaminating spray penetrated through the outer clothing to the underwear and skin.

Table 6
Experiment 3: Viability Assay of 3/4-Inch-Diameter (2.85-cm) Control Sample Disks

Item	No. of Disks	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution					Viable Spores Recovered	
		Undiluted	10^{-2} X	10^{-4} X	10^{-6} X	10^{-8} X	Per Sample	Per Sq Cm
Gas mask	4		TNTC	65,84	0,0	0,0	7.5×10^{7}	6.6×10^{6}
Nyco hood	4		TNTC	136,95	4,0	0,0	1.2×10^{8}	1.0×10^{7}
Shirt front (random)	3		TNTC	20,20	0,0	0,0	2.0×10^{7}	2.4×10^{6}
Trousers back (random)	3		TNTC	39,31	0,0	0,0	3.5×10^{7}	4.1×10^{6}
Shirt back	2		30,37	0,0	0,0	0,0	3.5×10^{5}	(6.1×10^{4})*
Shirt back	2		TNTC	38,30	1,0	0,0	3.4×10^{7}	6.0×10^{6}
Trousers	4		TNTC	54,54	1,0	0,0	5.4×10^{7}	4.7×10^{6}
Right arm	3		TNTC	14,6	0,0	0,0	1×10^{7}	1.2×10^{6}
Left arm	3		TNTC	7,4	0,0	0,0	5×10^{6}	5.8×10^{5}
Right shoe	4		TNTC	40	90	200	4×10^{7}	3.5×10^{6}
Left shoe	4		TNTC	60	100	TNTC	6×10^{7}	5.3×10^{6}
Gloves	-							4.3×10^{6}†

*This pair of disks was covered by the skirt of the hood.
†Average of shirt and trousers.

Table 7
Experiment 3: Viability Assay of Disinfected Items By Drop-Plate Method

Item	Extraction Solution (ml)	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution					Viable Spores Recovered Per Item	Estimated Item Area (cm^2)	Viable Spores Recovered Per Sq Cm	Disinfection (%)
		Undiluted	10^{-2} X	10^{-4} X	10^{-6} X	10^{-8} X				
Mask eyepiece	500	TNTC W	200 W*	1,4 W	0		0	160	0	100
Mask can. holder	500	TNTC W	200 W	3,5 W	0		0	260	0	100
Mask diaphragm	500	TNTC W	200 W	3,4 W	0		0	140	0	100
Mask headharness	500	TNTC W	300 W	5,2 W	0		0	190	0	100
Hood	1000	TNTC W	200 W	0	0		0	1900	0	100
Shirt back sample	10	4	5	11	45	250	4×10^{2}	26	15	99.9998
Shirt back sample	10	1,0	0	0	0	0	1×10^{2}	26	3.8	99.99994
Whole shirt	1000	50	200 W	0	0		5×10^{5}	8500	60	99.999
Trousers left leg	10	TNTC	250	150	65	500	2.5×10^{6}	26	10^{5}	97.9
Trousers right leg	10	97,4	0	0	0	0	9.7×10^{3}	26	370	99.992
Whole trousers	1000	150	11,13	1,0	0	—	1.5×10^{6}	8500	120	99.997
Right arm	40	9	6	5	15	60	3.6×10^{3}	11	330	99.97
Left arm	40	66	8	0	5	21	2.6×10^{4}	11	2400	99.6
Right shoe sole	500	7					3.5×10^{4}	190	180	99.995
Right shoe heel	500	0					0	100	0	100.00
Right shoe upper	500	50	3,0				2.5×10^{5}	190	1300	99.96
Left shoe sole	500	12					6.0×10^{4}	190	320	99.994
Left shoe heel	500	12					6.0×10^{4}	100	600	99.99
Left shoe upper	500	85	3,1				4.2×10^{5}	190	2200	99.96
Gloves	500	100	2,0				5×10^{5}	650	770	99.98

*White contaminant on plates

Table 8
Experiment 3: Viability Assay of Disinfected Items By Membrane Filter Method

Item	Extraction Solution (ml)	Plate Counts for Various Volumes of the Undiluted Extract Solution			Viable Spores Recovered Per Item	Estimated Item Area (cm^2)	Viable Spores Recovered Per Sq Cm	Disinfection (%)
		1 ml	10 ml	100 ml				
Mask eyepiece	500	40*	100	224	20000	160	125	99.998
Mask canister holder	500	4	8	80	2000	260	8	99.9999
Mask diaphragm	500	10	75	448	5000	140	36	99.9995
Mask headharness	500	45	70	448	22000	190	120	99.998
Hood	1000	224	TNTC	TNTC	2.2×10^5	1900	120	99.999
Whole shirt	1000	150	900	TNTC	1.5×10^5	8500	18	99.9996
Whole trousers	1000	TNTC	TNTC	TNTC	—	8500	—	—
Right shoe sole	500	71	448	TNTC	3.5×10^4	190	180	99.995
Right shoe heel	500	24	50	TNTC	1.2×10^4	100	120	99.997
Right shoe upper	500	560	TNTC	TNTC	2.8×10^5	190	1500	99.96
Left shoe sole	500	50	672	—	2.5×10^4	190	130	99.998
Left shoe heel	500	85	672	TNTC	4.2×10^4	100	420	99.992
Left shoe upper	500	448	TNTC	TNTC	2.2×10^5	190	1200	99.98
Gloves	500	TNTC	TNTC	TNTC	—	—	—	—

*Underlined data are those used in calculating the disinfection efficiency (last column).

Experiment 4

Experiment 4 was a repetition of Experiment 3, with a different uniform, a slightly different style of shoes, and the addition of underwear disinfection. The all-nyco uniform of the previous experiment was replaced by a composite of nyco hood, cotton sateen shirt, and wool flannel trousers.

The results are shown in Tables 9 through 11. Although the disinfection effectiveness throughout the experiment was somewhat less than in Experiment 3, it is considered to be highly satisfactory. The only exception is the wool flannel trousers, for which the disinfection effectiveness was a marginal 98.7 percent. This difficulty in disinfection of wool had been observed before, but earlier NRL data and those of Experiments 5 and 6, show that wool flannel or Melton cloth can be disinfected by a sufficiently forceful spray of SLASH.

The environmental factors measured in this experiment were: air temperature, 16°C (61°F); relative humidity, 72 to 80 percent; and wind speed, 2 to 4 mph. These resulted in a clothing surface temperature, after disinfection, of 18°C (64°F) on the shady side and 20°C (68°F) in the sun. It is interesting to note that the clothing surface temperature remained well above the wet-bulb temperature of 14°C (57°F). (It is this temperature differential which indicates both a substantial heat flow outward and the capacity for body cooling provided by a wetted outer garment.) The SLASH temperature in the tank during disinfection was 18°C (65°F).

Table 9
Experiment 4: Viability Assay of 3/4-Inch-Diameter (2.85-cm^2) Control Sample Disks

Item	No. of Disks	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution					Viable Spores Recovered	
		Undiluted	10^{-2} X	10^{-4} X	10^{-6} X	10^{-8} X	Per Sample	Per Sq Cm
Gas mask	2		TNTC	6,7			6.5×10^6	1.1×10^6
Nyco hood	2		TNTC	35,29			3.2×10^7	5.6×10^6
Shirt front (random)	2		TNTC	11,15			1.3×10^7	2.3×10^6
Shirt back (random)	2		TNTC	18,26			2.2×10^7	3.9×10^6
Trousers (random)	2		TNTC	19,20			2.0×10^7	3.5×10^6
Trousers (sample area)	2		TNTC	3,3			3.0×10^6	5.3×10^5
Right arm	2		TNTC	3,6			4.5×10^6	7.9×10^5
Left arm	2		203,241	3,3			3.0×10^6	5.3×10^5
Right shoe	2		TNTC	29,37			3.3×10^7	5.8×10^6
Left shoe	2		TNTC	33,36			3.4×10^7	6.0×10^6
Gloves	—		—	—			Av of shirt, trousers	2.6×10^6
Underwear	—		—	—			—	—

Table 10
Experiment 4: Viability Assay of Disinfected Items by Drop-Plate Method

Item	Extraction Solution (ml)	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution					Viable Spores Recovered Per Item	Estimated Item Area (cm^2)	Viable Spores Recovered Per Sq Cm	Disinfection (%)
		Undiluted	10^{-2} X	10^{-4} X	10^{-6} X	10^{-8} X				
Mask eyepiece	500	9,6	0,0	0,0			3.8×10^{4}	160	240	99.98
Mask can. holder	500	0,0	0,0	0,0			0	260	0	100.00
Mask diaphragm	500	0,0	0,0	0,0			0	140	0	100.00
Mask headharness	500	1,2	0,0	0,0			7.5×10^{3}	190	40	99.996
Hood	1000	91,81	6,6	0,9			8.6×10^{5}	1900	450	99.992
Shirt back sample	10	70,78	0,0	0,0			7.4×10^{3}	26	280	99.993
Shirt back sample	10	80,85	2,2	6,12			8.2×10^{3}	26	320	99.992
Whole shirt	1000	TNTC	37,37	0,0			3.7×10^{7}	8500	4400	99.9
Trousers sample	10	TNTC	15,18	0,0			1.6×10^{5}	26	6200	98.8
Trousers sample	10	TNTC	12,13	0,0			1.2×10^{5}	26	4800	99.1
Whole trousers	1000	TNTC	225,211	28,41			2.2×10^{8}	8500	26000	98.7
Right arm	40	188,170	1,3	0,0			7.2×10^{4}	11	6500	99.2
Left arm	40	2,0	0,0	0,0			4.0×10^{2}	11	3600	99.3
Right shoe sole	500	30,32	2,0	0,0			1.6×10^{5}	190	340	99.99
Right shoe heel	500	81,84	2,0	0,0			4.1×10^{5}	100	4100	99.93
Right shoe upper	500	105,105	1,1	0,0			5.2×10^{5}	190	2700	99.95
Left shoe sole	500	16,22	0,1	0,0			9.5×10^{4}	190	500	99.992
Left shoe heel	500	8,7	0,0	0,0			3.8×10^{4}	100	380	99.994
Left shoe upper	500	61,61	2,1	0,0			3.0×10^{5}	190	1600	99.97
Gloves	500	90,69	0,1	0,0			4.2×10^{5}	650	650	99.98
Underwear, 2 pcs	1000	45,70	0,0	0.0			5.8×10^{5}	8500	68	—

Table 11
Experiment 4: Viability Assay of Disinfected Items By Membrane Filter Method

Item	Extraction Solution (ml)	Plate Counts for Various Volumes of the Undiluted Extract Solution			Viable Spores Recovered Per Item	Estimated Item Area (cm^2)	Viable Spores Recovered Per Sq Cm	Disinfection (%)
		1 ml	10 ml	100 ml				
Mask eyepiece	500	126	TNTC		63,000	160	390	99.96
Mask canister holder	500	1	33		16,000	260	62	99.994
Mask diaphragm assy.	500	7	31		3,500	140	25	99.998
Mask headharness	500	16	106		8,000	190	42	99.996
Hood	1000	672	TNTC		670,000	1900	350	99.994
Whole shirt	1000	TNTC	TNTC		—	8500	—	—
Whole trousers	2000	TNTC	TNTC		—	8500	—	—
Right shoe sole	500	224	TNTC		110,000	190	580	99.99
Right shoe heel	500	336	TNTC		170,000	100	1700	99.97
Right shoe upper	500	672	TNTC		340,000	190	1800	99.97
Left shoe sole	500	120	TNTC		60,000	190	320	99.995
Left shoe heel	500	60	560		30,000	100	300	99.995
Left shoe upper	500	336	TNTC		170,000	190	840	99.99
Gloves	500	336	TNTC		170,000	650	240	99.991
Underwear, 2 pcs	1000	336	TNTC		340,000	8500	40	—

The actual effectiveness of underwear disinfection cannot be determined, because no controls were obtained for the degree of contamination. However, it is worth noting that the 68 residual spores per square centimeter on the underwear after disinfection were only 1.5 percent of the residual spores on the shirt, and 0.25 percent of those on the trousers. That is, the final residual contamination of the underwear was of the order of 1 percent that of the outer clothing. It also may be useful to refer the residual contamination on the underwear directly to the original contamination on the outer clothing. In this specialized sense, the effectiveness of underwear disinfection was 99.9998 percent.

Experiment 5

Tables 12 and 13 give the results of Experiment 5. A very extensive series of controls was used, including eight nyco disks, four stainless steel squares, and duplicates of the 28 different material samples which were decontaminated. In addition, the typewriter and electric drill were exposed for control data only. Since only one of each of these items was available, they were exposed as controls in one experiment, and disinfected in the next. The practice of a separate control for each disinfection sample was followed because of the lack of prior information on the uniformity of contamination to be expected on materials of widely differing composition and texture. The mean contamination level of the nyco controls was 5.85×10^3 spores per square centimeter, with a standard deviation of 1.79×10^3, or 31 percent, and an average deviation from the mean of 1.48×10^3. For the 28 construction materials, including the radio and the average of two aluminum crevice fixtures, but excluding the typewriter and electric drill, the mean contamination level was 6.9×10^3 spores per square centimeter, with a standard deviation of 3.6×10^3, or 52 percent, and an average deviation from the mean of 3.7×10^3. If the very large deviation of the asphalt shingle were excluded, the standard deviation would be reduced to 2.6×10^3, or 38 percent, and the average deviation to 2.3×10^3.

From the standpoint of deposition theory it would be of interest to attempt a correlation of the degree of contamination of the samples versus their surface texture. Unfortunately this cannot be done with accuracy because of the perturbing effect of the probably varying efficiency of spore removal from the different materials when shaken in the extraction solution. That is, the measured degree of contamination of the controls is actually the product of two factors: the true degree of contamination and the efficiency of spore removal. However, some progress can be made. One can first postulate that all materials are contaminated with precisely the same number of spores per unit area, as would probably occur in pure sedimentation, and assume that all variations are attributable to variations in ease of extraction of the spores. There are indications that the postulate is at least partly valid. The comparatively smooth and impermeable materials, such as the steel and plastic roofings, the ceramic tile, the hardboard, and the aluminum crevice fixtures, all show spore recoveries well above the average (Table 12), whereas a number of smooth but porous materials, such as the wood products, give below-average recoveries. The opposite postulate, that differences in contamination are solely due to actual differences in aerosol deposition on materials of different surface texture, also shows indications of validity. Of the four materials with the highest spore recovery, the asphalt shingle and concrete block have a very rough and granular surface, and the other two — the corrugated roofings — may be considered to have roughnesses in the form of the corrugations. It appears, therefore, that both postulates are partly correct.

Table 12
Experiment 5: Viability Assay of Control Contamination Samples

Item	Area (cm^2)	Extraction Solution (ml)	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution				Viable Spores Recovered	
			Undiluted	0.1 X	0.01 X	0.001 X	Per Item	Per Sq Cm
Nyco disk on typewriter	2.8	10	TNTC	13,22	0,0	0,0	1.8×10^4	6.4×10^3
Nyco disk on radio	2.8	10	TNTC	13,26	1,0	0,0	2.0×10^4	7.2×10^3
Nyco disk on floor, back	2.8	10	TNTC	11,13	1,0	0,0	1.2×10^4	4.3×10^3
Nyco disk on floor, front	2.8	10	TNTC	11,22	1,1	0,0	1.6×10^4	5.7×10^3
Nyco disk on floor, right	2.8	10	119,102	15,14	0,0	0,0	1.1×10^4	3.9×10^3
Nyco disk on floor, left	2.8	10	98,101	8,12	0,0	0,0	1.0×10^4	3.6×10^3
Nyco disk on floor, back ctr	2.8	10	TNTC	16,19	1,1	0,0	1.8×10^4	6.4×10^3
Nyco disk on floor, back ctr	2.8	10	TNTC	27,24	3,1	0,0	2.6×10^4	9.3×10^3
Stainless steel square, left front	2	10	TNTC	14,20	1,0	0,0	1.7×10^4	8.5×10^3
Stainless steel square, right front	2	10	TNTC	32,18	3,1	0,0	2.5×10^4	1.2×10^4
Stainless steel square, right center	2	10	TNTC	13,24	1,0	0,0	1.8×10^4	9×10^3
Stainless steel square, rear center	2	10	123,103	12,4	3,1	0,0	1.1×10^4	5.5×10^3
Radio	200	100	TNTC	116,122	9,10	1,1	1.2×10^6	6.0×10^3
Electric drill	560	200	TNTC	TNTC	79,80	15,7	1.6×10^7	2.9×10^4
Typewriter	1200	1000	TNTC	103,120	13,17	0,0	1.1×10^7	9.2×10^3
Manila rope	55	50	TNTC	48,41	26,19	0,0	2.2×10^5	4.0×10^3
Wire rope	10	50	81,67	6,3	2,1	0,0	3.7×10^4	3.7×10^3
Nylon rope	20	50	TNTC	30,21	3,2	0,0	1.3×10^5	6.5×10^3
Asphalt shingle	60	50	TNTC	TNTC	21,29	1,3	1.2×10^6	2.0×10^4
Tarpaper roofing	60	50	TNTC	51,55	7,7	1,0	2.6×10^5	4.3×10^3
Corrugated galvanized steel roofing	75	50	TNTC	120, —	17,16	1,1	8.0×10^5	1.1×10^4
Corrugated rigid plastic roofing	85	50	TNTC	TNTC	26,10	1,0	9.0×10^5	1.1×10^4
Canvas tarpaulin, impregnated	70	50	TNTC	110,113	7,15	7,9	5.6×10^5	8.0×10^3

(Table continues)

Table 12 (Continued)

Item	Area (cm^2)	Extraction Solution (ml)	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution				Viable Spores Recovered	
			Undiluted	0.1 X	0.01 X	0.001 X	Per Item	Per Sq Cm
Plywood	60	50	TNTC	29,30	4,5	1,0	1.5×10^5	2.5×10^3
Painted plywood	60	50	TNTC	74,64	6,8	1,0	3.5×10^5	5.8×10^3
End-grain lumber	45	50	TNTC	23,22	2,1	0,0	1.1×10^5	2.4×10^3
Weathered lumber	50	50	TNTC	36,38	10,6	2,0	1.8×10^5	3.6×10^3
Smooth concrete (sidewalk)	40	50	TNTC	62,78	9,4	1,0	3.5×10^5	8.8×10^3
Rough concrete (roadway)	50	50	TNTC	67,71	11,7	1,0	3.5×10^5	7.0×10^3
Concrete block	40	50	TNTC	109,91	8,6	1,0	5.0×10^5	1.2×10^4
Painted concrete block	55	50	TNTC	51,61	7,4	2,0	2.8×10^5	5.1×10^3
Brick	50	50	TNTC	30,33	5,2	0,0	1.6×10^5	3.2×10^3
Asphalt paving	50	50	TNTC	40,52	49,46	0,0	2.3×10^5	4.6×10^3
Vinyl floor tile	50	50	TNTC	73,62	28,12	0,0	3.4×10^5	6.8×10^3
Asphalt tile	70	50	TNTC	71,80	16,16	11,4	3.8×10^5	5.4×10^3
Corrugated rubber matting	55	50	TNTC	53,55	3,1	1,1	2.7×10^5	4.9×10^3
Glazed ceramic tile	120	50	TNTC	TNTC	27,15	3,0	1.1×10^6	9.2×10^3
Masonite hardboard	50	50	TNTC	105,92	13,9	1,1	4.9×10^5	9.8×10^3
Gypsum board	55	50	TNTC	55,62	12,5	1,0	2.9×10^5	5.3×10^3
Insulating board (beaverboard)	40	50	TNTC	36,37	7,3	2,0	1.8×10^5	4.5×10^3
Acoustic ceiling tile	60	50	TNTC	100,77	14,3	1,0	4.4×10^5	7.4×10^3
Fabricated aluminum crevice	35	50	TNTC	60,50	3,1	1,0	2.8×10^5	8.0×10^3
Fabricated aluminum crevice	35	50	TNTC	60,58	11,12	0	3.0×10^5	8.6×10^3
Average of the 28 construction materials, excluding typewriter and drill							4.2×10^5	6.9×10^3

Table 13
Experiment 5: Viability Assay and Disinfection Effectiveness

Item	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution				Disinfectant Used	Viable Spores Recovered Per Item, P = 0.95	Disinfection Effectiveness (%)
	Undiluted	0.1 X	0.01 X	0.001 X			
Radio	0,0	0,0	0,0	0,0	SLASH	<3000	99.8+*
Manila rope	0,0	0,0	0,0	0,0	SLASH	<1500	99.3+
Wire rope	0,0	0,0	0,0	0,0	SLASH	<1500	95.9+
Nylon rope	0,0	0,0	0,0	0,0	SLASH	<1500	98.8+
Asphalt shingle	0,0	0,0	0,0	0,0	ASH	<1500	99.9+
Tarpaper roofing	0,0	0,0	0,0	0,0	ASH	<1500	99.4+
Corrug. galv. steel roofing	0,0	1,0	1,1	0,0	ASH	<1500	99.8+
Corrug. rigid plastic roofing	0,0	0,0	0,0	0,0	ASH	<1500	99.8+
Canvas tarpaulin, impregnated	0,0	0,0	0,0	0,0	ASH	<1500	99.7+
Plywood	0,0	0,0	0,0	0,0	ASH	<1500	99.0+
Painted plywood	0,0	0,0	0,0	0,0	ASH	<1500	99.6+
End-grain lumber	1,0	0,0	1,0	0,0	ASH	<1500	98.6+
Weathered lumber	0,0	0,0	0,0	0,0	ASH	<1500	99.2+
Smooth concrete (sidewalk)	0,0	0,0	0,0	0,0	ASH	<1500	99.6+
Rough concrete (roadway)	0,0	0,0	0,0	0,0	ASH	<1500	99.6+
Concrete block	0,0	0,0	0,0	0,0	ASH	<1500	99.7+
Painted concrete block	0,0	0,0	0,0	0,0	ASH	<1500	99.5+
Brick	0,0	0,0	0,0	0,0	ASH	<1500	99.1+
Asphalt paving	0,0	0,0	0,0	0,0	ASH	<1500	99.4+
Vinyl floor tile	7,0	0,0	0,0	0,0	ASH	<1750	99.95
Asphalt tile	0,0	0,0	0,0	0,0	ASH	<1500	99.6+
Corrugated rubber matting	0,0	0,0	0,0	0,0	ASH	<1500	99.4+
Glazed ceramic tile	0,0	0,0	0,0	0,0	ASH	<1500	99.86+
Masonite hardboard	0,0	0,0	0,0	0,0	ASH	<1500	99.7+
Gypsum board	0,0	0,0	1,0	0,0	SLASH	<1500	99.5+
Insulating board (beaverboard)	0,0	0,0	0,0	0,0	SLASH	<1500	99.2+
Acoustic ceiling tile	0,0	0,0	0,0	1,0	SLASH	<1500	99.7+
Fabricated aluminum crevice	0,0	0,0	0,0	0,0	SLASH	<1500	99.5+
Fabricated aluminum crevice	0,0	0,0	0,0	0,0	SLASH	<1500	99.5+

*95 percent probability of better than 99.8 percent decontamination effectiveness. If all samples are regarded as identical replicates, the spore count per item is 55 and decontamination effectiveness becomes: $100 - [(55 \times 100)/(4.2 \times 10^5)] = 99.99+$ percent.

As to disinfection effectiveness of the ASH and SLASH systems the spore recoveries after disinfection were zero in almost every case.* Although this is very satisfactory in view of the several hundred thousand spores with which most samples were originally contaminated, a problem of computation occurs. This arises because only a small aliquot, 0.1 ml, of the 50 ml of extraction solution is actually assayed. The 50 ml might contain some spores, even though none are found in the 0.1 ml aliquot. A mathematical treatment applicable to this situation has been described in the first report of this series (11). The procedure yields decontamination effectivenesses which, to a probability of 95 percent, are better than the tabulated numbers. The calculation is

$$P = 1 - e^{-\lambda V},$$

where P is the probability, set at 0.95 in this case, of finding at least one spore in V; λ is the calculated average number of spores present per milliliter of the extraction solution, or its dilution, which is analyzed; and V is the volume of the analyzed aliquot of the same solution. Since V was fixed at 0.1 ml in all cases, λ is uniformly calculated to be less than 30 spores per milliliter at a probability of 95 percent. In the case of the radio, for which 100 ml of extraction solution was used, the total spores were calculated at less than 30×100, or 3000. With the control number of spores, 1.2×10^6, the calculated decontamination effectiveness is greater than 99.75, or 99.8, percent. How much greater is, of course, unknown. The large number of samples with zero spores actually recovered tends to reinforce the supposition that the spores actually present after disinfection may have been considerably less than the tabulated figures. This concept can be given mathematical form by regarding all samples as essentially replicates, and by lumping all aliquots into a single large aliquot assumed to be taken from the 50 ml of extraction solution of a single sample. Thus V becomes 28×0.1 ml and λ is calculated to be 1.1 spores per milliliter of extraction solution. The 50 ml of extraction solution thus contained 55 spores. With the average deposition per item of 4.2×10^5 spores, the disinfection effectiveness is calculated to be 99.99+ percent.

Experiment 6

Tables 14 through 17 present the results of Experiment 6. Again, the recovery of spores from disinfected items was generally zero. Spores were recovered from the typewriter, the dirty finned-tube section, the loamy soil, one of three nyco samples disinfected with 0.25 percent SLASH, nyco saturated with motor oil, wool saturated with vegetable oil, wool saturated with lubricating grease, and nyco sprayed through two intervening layers of nyco. Nevertheless, the disinfection effectiveness in these difficult cases, was respectively 99.6, 97, 99.96, 99.94, 99.4, 99.7, 99.94, and 90.7 percent.

The adaptability of hypochlorous acid to vapor phase disinfection was explored very briefly. Sixteen 5/8-inch disks were punched out of nyco fabric previously exposed in the aerosol chamber. Twelve weighing bottles were equipped with nichrome wire hooks cemented to the underside of the glass stopper (Fig. 9). A nyco disk was hung from each hook (the bottom edge of each disk being suspended a few millimeters above the liquid level), and the remaining four disks were used as controls. By timing both the addition of 2 ml of ASH to each bottle and the removal of the disks, the disks were exposed for periods of 1 to 32 minutes. The results, shown in Table 17, appear to be sufficiently good to justify further study. The disks which were completely disinfected are assumed to have accidentally touched the ASH surface and absorbed ASH by capillary action, although no such contact was actually observed.

*Instances of finding occasional single colonies could have been due to aerial contamination of plates, since other experimenters in the area were using the same organism.

Table 14
Experiment 6: Viability Assay of Control Contamination Samples

Item	Area (cm^2)	Extraction Solution (ml)	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution				Viable Spores Recovered	
			Undiluted	0.1 X	0.01 X	0.001 X	Per Item	Per Sq Cm
Cloth disk on typewriter	2.8	10	TNTC	31,34	5,9	6,0	3.2×10^5	1.1×10^4
Cloth disk near typewriter	2.8	10	TNTC	26,42	2,2	1,0	3.4×10^4	1.2×10^4
Cloth disk on floor, right rear	2.8	10	TNTC	32,35	3,7	0,0	3.4×10^4	1.2×10^4
Cloth disk on floor, right center	2.8	10	TNTC	26,16	3,2	0,0	2.1×10^4	7.5×10^3
Cloth disk on floor, right center	2.8	10	TNTC	32,25	2,4	0,0	2.8×10^4	1.0×10^4
Cloth disk on floor, right front	2.8	10	TNTC	46,44	2,4	1,0	4.5×10^4	1.6×10^4
Cloth disk on floor, right front	2.8	10	TNTC	40,30	3,2	0,0	3.5×10^4	1.2×10^4
Cloth disk on floor, front center	2.8	10	TNTC	20,22	2,3	0,0	2.1×10^4	7.5×10^3
Radio	200 est	100	TNTC	116,122	9,10	1,1	1.2×10^6	6.0×10^3
Electric drill	560 est	200	TNTC	TNTC	79,80	15,7	1.6×10^7	2.9×10^4
Typewriter	1200 est	1000	TNTC	103,120	13,17	0,0	1.1×10^7	9.2×10^3
Finned-tube section, clean	60 est	50	TNTC	TNTC	15,16	5,1	8.0×10^5	1.3×10^4
Finned-tube section, dirty	60 est	50	TNTC	50, —	9,0	1,0	2.5×10^5	4.2×10^3
Hairy leaf	60 est	30	TNTC	TNTC	45,40	3,6	1.3×10^6	2.2×10^4
Smooth leaf	60 est	10	TNTC	TNTC	70,87	2,5	7.8×10^5	1.3×10^4
Soil, loam	45	50	TNTC	TNTC	20,21	1,0	1.0×10^6	2.2×10^4
Soil, sandy	45	50	TNTC	TNTC	16,17	2,0	8.5×10^5	1.9×10^4
Nyco	25	10	TNTC	TNTC	48,41	6,5	4.4×10^5	1.8×10^4
Cotton sateen	25	10	TNTC	TNTC	56,68	5,7	6.2×10^5	2.5×10^4
Quardel-treated cotton sateen	25	10	TNTC	TNTC	52,42	3,8	4.7×10^5	1.9×10^4
Wool	25	10	TNTC	TNTC	39,47	3,2	4.3×10^5	1.7×10^4
Nyco saturated with motor oil	25	10	TNTC	TNTC	26,28	2,2	2.7×10^5	1.1×10^4
Nyco saturated with vegetable oil	25	10	TNTC	TNTC	23,26	2,2	2.4×10^5	1.0×10^4
Nyco saturated with lubricating grease	25	10	TNTC	TNTC	62,74	6,11	6.6×10^5	2.6×10^4
Wool saturated with motor oil	25	10	TNTC	TNTC	23,21	2,1	2.2×10^5	8.8×10^3
Wool saturated with vegetable oil	25	10	TNTC	TNTC	13,21	2,4	1.7×10^5	6.8×10^3
Wool saturated with lubricating grease	25	10	TNTC	TNTC	32,21	1,1	2.6×10^5	1.0×10^4

Table 15
Experiment 6: Calculated Contamination of Samples Based on Average Contamination of Adjacent Cloth Disks

Item	Area (cm^2)	Estimated Viable Spores Recovered	
		Per Sq Cm	Per Item
Plywood	60	1.1×10^4	6.6×10^5
Concrete block	40	1.1×10^4	4.4×10^5
Weathered lumber	50	1.1×10^4	5.5×10^5
Insulating board	40	1.1×10^4	4.4×10^5
Painted plywood	60	1.1×10^4	6.6×10^5
Gypsum board	55	1.1×10^4	6.0×10^5
Asphalt paving	50	1.1×10^4	5.5×10^5
Painted concrete block	55	1.1×10^4	6.0×10^5
Brick	50	1.1×10^4	5.5×10^5
Smooth concrete (sidewalk)	40	1.1×10^4	4.4×10^5
Rough concrete (roadway)	50	1.1×10^4	5.5×10^5
End-grain lumber	45	1.1×10^4	5.0×10^5
Masonite hardboard	50	1.1×10^4	5.5×10^5
Nyco	25	1.1×10^4	2.8×10^5

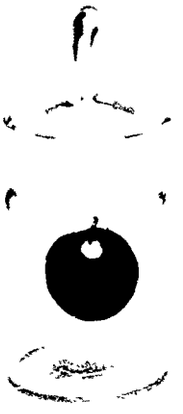


Fig. 9 - Arrangement used for vapor-phase disinfection of nyco disks with ASH

Table 16
Experiment 6: Viability Assay and Decontamination Effectiveness

Item	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution				Disinfectant Used	Viable Spores Recovered Per Item	Disinfection Effectiveness (%)
	Undiluted	0.1 X	0.01 X	0.001X			
Radio	0,0	0,0	—	—	SLASH	< 3000	99.8+
Radio	0,0	0,0	—	—	SLASH	< 3000	99.8+
Electric drill	0,0	0,0	—	—	SLASH	< 600	99.996+
Typewriter	5,4	2,1	—	—	SLASH	4.5×10^4	99.6
Finned-tube section, clean	0,0	0,0	—	—	SLASH	< 1500	99.8+
Finned-tube section, dirty	1,0	3,0	—	—	SLASH	7.5×10^3	97
Hairy leaf	0,0	0,0	—	—	ASH	< 600	99.95+
Smooth leaf	0,0	0,0	—	—	ASH	< 300	99.96+
Soil, loam	8,10	4,6	—	—	ASH	4.5×10^3	99.96
Soil, sand	0,0	0,0	—	—	ASH	< 1500	99.8+
Nyco	0,0	1,0	—	—	SLASH	< 300	99.93+
Nyco	4,1	1,0	—	—	25% SLASH*	2.5×10^2	99.94
Nyco	0,0	1,1	—	—	25% SLASH	< 300	99.93+
Nyco	0,0	0,0	—	—	25% SLASH	< 300	99.93+
Cotton sateen	0,0	0,0	—	—	SLASH	< 300	99.95+
Cotton sateen	0,0	0,0	—	—	SLASH	< 300	99.95+
Quardel-treated cotton sateen	0,0	0,0	—	—	SLASH	< 300	99.94+
Quardel-treated cotton sateen	0,0	0,0	—	—	SLASH	< 300	99.94+
Wool	0,0	0,1	—	—	SLASH	< 300	99.93+
Wool	0,0	0,1	—	—	SLASH	< 300	99.93+
Nyco saturated with motor oil	6,2	3,2	—	—	SLASH	1.5×10^3	99.4
Nyco saturated with vegetable oil	0,0	0,0	—	—	SLASH	< 300	99.88+
Nyco saturated with lubricating grease	0,0	0,0	—	—	SLASH	< 300	99.95+
Wool saturated with motor oil	0,0	0,0	—	—	SLASH	< 300	99.86+
Wool saturated with vegetable oil	5,5	1,0	—	—	SLASH	5.0×10^2	99.7
Wool saturated with lubricating grease	2,1	0,0	—	—	SLASH	1.5×10^2	99.94
Plywood	0,0	0,0	—	—	ASH	< 1500	99.8+
Plywood	0,0	0,0	—	—	ASH (STB)	< 1500	99.8+
Concrete block	0,0	0,0	—	—	ASH	< 1500	99.7+
Concrete block	0,0	0,0	—	—	ASH (STB)	< 1500	99.7+
Weathered lumber	0,0	0,0	—	—	ASH	< 1500	99.7+
Weathered lumber	0,0	0,0	—	—	ASH (STB)	< 1500	99.7+
Insulating board (beaverboard)	0,0	0,0	—	—	ASH	< 1500	99.7+
Insulating board (beaverboard)	0,0	0,0	—	—	ASH (STB)	< 1500	99.7+
Painted plywood	0,0	0,0	—	—	ASH	< 1500	99.8+
Painted plywood	1,0	0,0	—	—	ASH (STB)	< 1500	99.8+
Gypsum board	0,0	1,0	—	—	ASH	< 1500	99.7+
Gypsum board	0,0	0,0	—	—	ASH (STB)	< 1500	99.7+
Asphalt paving	0,0	0,0	—	—	ASH	< 1500	99.7+
Asphalt paving	0,0	0,0	—	—	ASH (STB)	< 1500	99.7+
Painted concrete block	0,0	0,0	—	—	ASH	< 1500	99.7+
Painted concrete block	0,0	0,0	—	—	ASH (STB)	< 1500	99.7+
Brick	0,0	0,0	—	—	ASH	< 1500	99.7+
Brick	0,0	0,0	—	—	ASH (STB)	< 1500	99.7+
Smooth concrete (sidewalk)	0,0	0,0	—	—	ASH	< 1500	99.7+
Smooth concrete (sidewalk)	0,0	0,0	—	—	ASH (STB)	< 1500	99.7+
Rough concrete (roadway)	0,0	1,1	—	—	ASH	< 1500	99.7+
Rough concrete (roadway)	0,0	0,0	—	—	ASH (STB)	< 1500	99.7+
End-grain lumber	0,0	0,0	—	—	ASH	< 1500	99.7+
End-grain lumber	0,0	0,0	—	—	ASH (STB)	< 1500	99.7+
Masonite hardboard	0,0	0,0	—	—	ASH	< 1500	99.7+
Masonite hardboard	0,0	0,0	—	—	ASH (STB)	< 1500	99.7+
Beaten nyco	0,0	0,0	—	—	SLASH	< 300	99.9+
Beaten nyco	0,0	0,0	—	—	SLASH	< 300	99.9+
Nyco disinfected through one layer	0,0	0,0	—	—	SLASH	< 300	99.9+
Nyco disinfected through two layers	—	31,22	—	—	SLASH	2.6×10^4	90.7

*Hypochlorite solution 25% of standard concentration, citrate concentration unchanged.

Table 17
Experiment 6: Vapor Phase Disinfection of Nyco Disks

Item	Treatment Time (min)	Plate Counts per 0.1 ml of Various Dilutions of the Extraction Solution			Viable Spores Per Disk	Av Viable Spores Per Disk	Disinfection Effectiveness (%)
		Undiluted	0.1 X	0.01 X			
Control disk	0	TNTC	20,22	3,2	2.1×10^4		
Control disk	0	TNTC	23,24	2,2	2.4×10^4	2.4×10^4	0
Control disk	0	TNTC	23,27	2,5	2.5×10^4		
Control disk	0	TNTC	30,25	3,4	2.8×10^4		
Treated disk	1	TNTC	26,31	2.4		2.4×10^4	0
Treated disk	1	TNTC	17,23	1,1			
Treated disk	3	0,0	0,0	0,0		2.4×10^4	0
Treated disk	3	TNTC	22,26	2 0			
Treated disk	4	TNTC	24,28	1,4		2.9×10^4	0
Treated disk	4	TNTC	40,23	4,5			
Treated disk	8	TNTC	15,24	3,1		1.9×10^4	21
Treated disk	8	TNTC	18,18	1,0			
Treated disk	16	TNTC	7,8	2,0		1.1×10^4	54
Treated disk	16	TNTC	16,12	1,5			
Treated disk	32	0,0	0,0	0,0		1.3×10^3	95
Treated disk	32	11,15	1,1	1,1			

The results of this experiment which are probably most noteworthy are (a) satisfactory decontamination of two civilian radios, an electric drill, and a typewriter, with no adverse effects on the prompt operation of these devices and (b) semisuccessful use of SLASH at only 1/4 the usual concentration of hypochlorous acid, the entirely successful replacement of calcium hypochlorite in ASH by supertropical bleach, and the expansion of the use of ASH/SLASH to a wide selection of materials which might be expected to be decontamination-resistant, including oily and greasy fabrics, water-repellent fabrics, soils, and leaves.

Corrosion-Sensitive Devices and Materials

The manual typewriter used for a contamination control and for disinfection in Experiments 5 and 6 was a severe test for the corrosion properties of SLASH. Although the SLASH treatment did not affect prompt operation of the typewriter, later operation was affected. After evaporation of the alcohol used in the dewatering treatment, the typewriter was shipped to NRL in an airtight bag. On arrival, 2 weeks later, the machine showed sufficient rust to disallow normal smooth operation. Insufficient data exist to compare precisely the corrosiveness of SLASH relative to that of pure water. The available facts suggest, however, that it was not the SLASH treatment which finally disabled the typewriter but rather the complete removal of oil films by the SLASH detergent followed by 2 weeks of exposure to a humid atmosphere. Were such a test to be repeated, water removal should be assured by oven drying, after which all bare metal parts should be sprayed with an oil mist, or the machine packaged with desiccant pending future attention. In any case the performance of a BW/CW decontaminant on a typewriter can be regarded as a highly sensitive indicator of corrosivity. This follows from the fact that large numbers of bare steel parts plus the even larger number of pivots and sliding surfaces are all highly susceptible to corrosion when oil and grease is removed.

Electrical and Electronic Devices

Two identical personal AM/FM transistor radios and one 1/4-inch electric drill were studied for decontamination susceptibility and possible decontamination damage in Experiments 5 and 6. In Experiment 5 one radio and the electric drill acted as controls only; i.e., they were shaken well in the extraction solution, rinsed under a tapwater stream, and sprayed with isopropyl alcohol, which was allowed to evaporate. In the same experiment the other radio was decontaminated with SLASH after the cover was removed to expose circuit components. Rinsing with water and spraying with alcohol followed as for the control radio. In Experiment 6, both radios and the electric drill were decontaminated, rinsed, and sprayed with alcohol. In both experiments no visible spores were recovered, and all three devices remained operational with no perceptible degradation in performance. Both radios did show, however, a slight attack on the plastic window covering the tuning dials. This was attributed to the solvent effect of the isopropyl alcohol. It should also be mentioned that one step was taken to protect the radios from the repeated exposure to liquids in the cycle of SLASH spray, shaking in extraction solution, rinsing in tapwater, and alcohol spray. The step was to seal with adhesive tape the several small holes in the plastic covers of the variable condensers. This was considered advisable because of the anticipated difficulty of removing water trapped under the covers. In some cases the tape failed to keep out the water, and the condensers had to be blown out with air.

The decontamination experience with radios and an electric drill in the NBL/NRL studies, plus preparatory tests at NRL involving immersion of the same items in water, suggest that what difficulties may exist in the SLASH treatment of electrical and electronic gear are not problems with SLASH as such, or problems of contact with water, but are rather problems of water removal. The fact that this was successfully accomplished several times with civilian radios indicates that the task would be even simpler with military electronic hardware, which is designed and packaged for a much higher degree of water resistance than are low-priced civilian radios.

Difficultly Accessible Surfaces

The ability of SLASH to reach and kill spores in difficultly accessible recesses was challenged by the aluminum crevice device and by the finned-tube section. These surfaces were satisfactorily disinfected when they were free from oil and dirt. The finned-tube section which had not been precleaned, i.e., which retained its normal deposit of atmospheric dirt, was only 97 percent disinfected. The problem here is believed to be formally comparable to those of disinfection of woolen clothing and of disinfection of two layers of clothing in one spraying; in all cases disinfection efficiency can be expected to improve with the use of high-pressure, high-volume spray equipment.

Natural Materials

The reasonably successful disinfection of two kinds of soil and two types of foliage indicate that no serious problems need be anticipated in the application of the ASH/SLASH system to natural materials.

ASH, Regular and STB

Experiment 6 demonstrated unequivocally that highly effective disinfection can be accomplished either with ASH formulated in the usual way or with supertropical bleach (STB) as the source of hypochlorite. In fact, there is no reason to suppose that the two

solutions are not precisely equal in effectiveness. The qualitative equivalence of 70-percent calcium hypochlorite and STB as hypochlorite sources for ASH was anticipated, but the quantitative demonstration was nevertheless important. The U.S. armed forces have very large quantities of STB in storage for possible BW/CW decontamination needs. In military manuals, STB, at concentrations of 7 to 40 percent (2.3 to 13 percent available chlorine), is recommended as a BW countermeasure for use on terrain; however, it is not recommended in these manuals for use on personnel (12,13). The ASH/SLASH system can be used on almost any surface requiring disinfection, and assures much greater effectiveness than results from the usual STB slurries or solutions. Heretofore, however, ASH/SLASH could be prepared only from 70-percent calcium hypochlorite. Now it is clear that the very effective and innocuous ASH/SLASH solutions can be made from the large stocks of STB available to the military and, presumably, to civil defense organizations.

The formulas for preparing 1 liter of ASH containing 0.2 percent hypochlorous acid from calcium hypochlorite and STB are as follows:

Hypochlorite Source	Quantity of Hypochlorite	Quantity of NBC Detergent	Quantity of NaH_2PO_4 Monohydrate
Calcium hypochlorite, 70%	3 g	5 g	4.5 g
STB	7 g	5 g	7 g

GENERAL DISCUSSION

This section of the report will highlight what may be considered to be the principal accomplishments of the NBL/NRL experiments and suggest areas in which further work is desirable.

Man and Clothing

Experiments 3 and 4 show that a man and his clothing can be disinfected, after contamination with highly refractory spores, by spraying with SLASH from a low-pressure hand sprayer, with no harm to subject or clothing. The results of these experiments are summarized in Tables 18 and 19. In these tables the spore recoveries by the membrane filter method are generally favored for disinfection effectiveness calculations over those obtained by the drop-plate method. This choice was made on the basis of the larger volumes of extraction solution plated in the former method — 1, 10, or occasionally 100 ml — in comparison with the 0.1 ml or decimal fraction thereof plated in the latter method.

The data of Tables 18 and 19 are further summarized in Table 20. It is clear that disinfection was consistently less complete in Experiment 4, whether or not the wool trousers of Experiment 4 are considered. This consistent difference in the results of the two experiments is attributed to the previously mentioned partial clogging of the wire screen in the hypochlorite line. As mentioned previously, one result of this malfunction was a 48-second half life rather than the approximately 100 seconds expected. Probably more influential in reducing disinfection effectiveness was the accompanying reduction in the initial hypochlorite concentration and in the total flow rate.

Table 18
Summary of SLASH Disinfection of Man and His Clothing in Experiment 3

Item Disinfected	Viable Spores Found Per Sq Cm			Disinfection (%)	Reduction In Contamination (factors of 10)
	Before Disinfection (Controls)	After Disinfection			
		Drop-Plate Assay	Filter Assay		
Gas mask					
Eyepiece		0	125		
Canister holder		0	8		
Diaphragm assy		0	36		
Headharness		0	120		
Gas mask average	6.6×10^6	0	67	99.999	5.0
Hood	1.0×10^7	0	120	99.999	4.9
Shirt	4.2×10^6	60	18	99.9996	5.4
Trousers	4.4×10^6	120	TNTC	99.997	4.6
Right shoe	3.5×10^6	490	600		
Left shoe	5.3×10^6	1040	580		
Shoe average	4.4×10^6	760	590	99.99	3.9
Gloves	4.3×10^6	770	TNTC	99.982	3.7
Right arm	1.2×10^6	330	—		
Left arm	5.8×10^5	2400	—		
Arm average	8.9×10^5	1360	—	99.85	2.8

Although the disinfection of the wool trousers in Experiment 4 was marginal at 98.7 percent, no such effect was observed in Experiment 6 when 3-inch squares of the same material were disinfected to better than 99.93 percent with SLASH. The difference in these two experiments, with regard to the disinfection of wool, is believed to be due largely to the spray and sprayer characteristics. Experiments 3 and 4 called for the complete spraying of a clothed man in a reasonably short period of time. The nozzle therefore was held sufficiently far from the subject (10 to 12 inches) to allow the spray to fan out and yield good coverage. In Experiment 6, only a 3-inch square of fabric was being sprayed. The nozzle therefore could be, and was, held about 3 or 4 inches from the substrate, giving the effects of a more concentrated and forceful spray. Corresponding results were noted in earlier NRL experiments in wool decontamination. Wool merely immersed in ASH was disinfected to an extent of 50 to 90 percent, but when suspended and sprayed with SLASH in exactly the manner of NBL/NRL Experiment 6, no viable spores were recovered.

The generally good results in the disinfection of oily and greasy cotton and wool in Experiment 6 may also be attributed, at least in part, to the concentrated spray pattern used. It was further observed in Experiment 6 that nyco could be well-disinfected when sprayed through an intervening layer of nyco and that 90.7 percent disinfection is attainable even when the spray must penetrate two layers of nyco to reach the contaminated fabric. In Experiments 3 and 4, however, the underwear under one layer of outer clothing was noticed to be incompletely wetted by the SLASH.

Table 19
Summary of SLASH Disinfection of Man and His Clothing in Experiment 4

Item Disinfected	Viable Spores Found Per Sq Cm			Disinfection (%)	Reduction In Contamination (factors of 10)
	Before Disinfection (Controls)	After Disinfection			
		Drop-Plate Assay	Filter Assay		
Gas mask					
Eyepiece		240	390		
Canister holder		0	62		
Diaphragm assy		0	25		
Headharness		40	42		
Gas mask average	1.1×10^6	61	130	99.99	3.9
Hood	5.6×10^6	450	350	99.994	4.2
Shirt	3.1×10^6	4400	—	99.9	2.8
Trousers	2.0×10^6	26000	—	98.7	1.9
Right shoe	5.8×10^6	1290	1300		
Left shoe	6.0×10^6	1620	520		
Shoe average	5.9×10^6	1460	910	99.98	3.8
Gloves	2.6×10^6	650	240	99.991	4.0
Underwear	—	68	40		
Right arm	7.9×10^5	6500	—		
Left arm	5.3×10^5	3600	—		
Arm average	6.6×10^5	5000	—	99.2	2.1

Table 20
Overall Summary of SLASH Disinfection of Man and His Clothing in Experiments 3 and 4

Experiment	Average Reduction in Contamination (factors of 10)		
	Clothing and Shoes		Skin of Arms
	Including Trousers	Excluding Trousers	
3	4.6	4.6	2.8
4	3.4	3.7	2.1

The only consistent and reasonable explanation of all of the above experimental data is that the spray force per unit area, and possibly the closely related liquid volume applied per unit area per unit time, are in fact important parameters in the disinfection of clothed men by SLASH. It appears, therefore, that for rapid disinfection of numbers of men, while maintaining an assured high level of effectiveness, spray devices should be selected or designed for the delivery of larger volumes per unit time at higher pressures than is feasible with the NRL two-tank device. Such a high-volume, high-pressure system may also permit the use of lower SLASH concentrations, as is suggested by the results of Experiment 6 using SLASH diluted fourfold.

Materials of Construction

Some 28 materials of construction were disinfected with ASH or SLASH in Experiment 5; effectiveness was very high in all cases. After rinsing with thiosulfate solution to neutralize any residual hypochlorite, 14 of these were rinsed, dried, and reused in duplicate in Experiment 6. Again, no viable spores were recovered after disinfection. Thus, 14 of the more important construction materials were decontaminated three times, and 14 others were decontaminated once. In every case the disinfection effectiveness was between very high and complete; the sensitivity of the overall system does not allow more precise definition of the degree of effectiveness. Metallic materials, painted* or bare, are not prominent in the list of construction-material samples. The reason is that these materials are inherently impermeable and nonabsorbent, and hence among the most easily decontaminated. Only when they are so formed or assembled that crevices and other difficultly accessible areas occur, or when they are coated with oil, grease, or dirt, do metallic items present decontamination difficulties. Areas of this nature were studied to a limited degree in Experiments 5 and 6 and are discussed in a following section. The corrosion of bare, unpainted metals also is a problem related to disinfection. This has been greatly reduced in ASH relative to older hypochlorite decontaminants, and is still lower in SLASH, but was not studied specifically in the NBL/NRL experiments. Useful indications were, of course, obtained in the disinfection of the radios, the electric drill, and the typewriter.

FUTURE WORK

This report represents, for the present at least, the final NRL involvement in laboratory studies on disinfection (or BW decontamination) as such. It is therefore appropriate to summarize future studies prerequisite to a fully developed, fully proven, fully ready ASH/SLASH system which will operate consistently with very high efficiency at low cost. Fourteen studies, or lines of effort, are listed below, together with brief comments.

1. An analysis of the feasibility and desirability of incorporating the ASH/SLASH system into military and civil public health and medical practice.

2. Larger-scale experiments on disinfection of clothed man. The objective would be to establish and prove procedures, determine quantities of materials and times required, and measure disinfection effectiveness for the SLASH treatment of groups of, say, 10 to 100 men. This effort presupposes the following one.

3. Development or selection of spray equipments, and associated reservoirs, mixers, and controls, to rapidly, effectively, and economically disinfect groups of men.

---

*In disinfecting painted materials, the paint is being disinfected, not the underlying material. Painted items of the same shape and surface arrangement would be expected to respond identically to ASH or SLASH disinfection regardless of the construction material under the paint.

4. Disinfection of the entire exterior and interior of buildings.

5. Disinfection of ships, vehicles, and aircraft. Injection of ASH/SLASH into shipboard washdown systems appears feasible.

6. Disinfection of weapons and ammunition.

7 Disinfection of a wide variety of electrical and electronic equipment.

8. Corrosion, and countermeasures for corrosion, of metals by ASH/SLASH. Although these disinfectants are low or very low in corrosivity, the substrates and conditions for which corrosion becomes a significant problem need to be defined more precisely, and anticorrosion measures need to be studied for use in the situations where their use is advisable. Such measures, for example, may be simple dewatering methods.

9. Deterioration of fabrics and cordage in contact with ASH/SLASH. That some degree of chemical action of ASH/SLASH on fabrics, cables, ropes, and lines occurs is likely for the most of the common fibers. The extent of the action and its effect as reflected in tensile strength and service life is the information needed. Serious difficulty is not anticipated in this area, but precise definition of the limits of usefulness of ASH/SLASH is desirable.

10. Physiological effects in the eyes and in passages affected by ingested disinfectants. The irritancy of ASH/SLASH on the skin and on raw tissue appears to be very low. Nevertheless, in the important area of physiological effects, precise and expert determinations are highly desirable. This should not overlook the effects of inhaled spray or vapor or the effects of eating food disinfected with ASH/SLASH. The latter involves the possible toxicity or other adverse effects of the presently undefined organic products of the citrate-hypochlorous acid reaction.

11. Cold-weather adaptations and effectiveness. Considerable work in this area has been done at Fort Detrick and NBL, and a small amount at NRL. All indicate that regular ASH/SLASH is highly effective at temperatures down to 0°C (32°F). At temperatures much below freezing, antifreeze additives are required, and the speed of action decreases very rapidly. Development of these parameters is required in operational terms, however.

12. Development of ASH/SLASH for CW decontamination. This is an obvious requirement, and the effort has been initiated at NRL, but it is listed here for completeness.

13. Doctrine for operational use of ASH/SLASH throughout the Navy. This can be begun now on the basis of present knowledge, and presuming the adaptation and use of present spraying or hosing equipment until more suitable equipment is available. A noteworthy proposal has been made by the NBC Defense School, Treasure Island, for the use of fire-fighting foam injectors to mix hypochlorite concentrates into hose streams.

14. Measurement of the characteristics of ASH/SLASH solutions when prepared in seawater. Any differences are expected to be negligible, but firm evidence is required.

SUMMARY AND CONCLUSIONS

1. SLASH neutral hypochlorite solutions are effective in disinfecting clothed men without noticeable irritation or clothing damage.

2. This effectiveness extends to clothing fabrics saturated with oil or grease and to nylon-cotton fabric covered with at least one layer of the same material.

3. A wide variety of exterior and interior construction materials are effectively disinfected with ASH or SLASH.

4. An electric drill and two AM/FM transistor radios have been disinfected by SLASH with no effect on performance.

5. In the very few cases of less than satisfactory disinfection the deficiency is attributed to insufficient spray pressure and flow rate, and correction of the deficiency is expected with the use of high-pressure, high-volume spray equipment.

RECOMMENDATIONS

1. It is recommended that further research and development be done in the ASH/SLASH system relative to metallic corrosion, fabric degradation, irritation and toxicity, cold weather formulations, and design or selection of spray equipments.

2. It is recommended that operational testing and evaluation be done in the ASH/SLASH system relative to the larger scale disinfection of clothed men, vehicles, ships, aircraft, weapons, ammunition, and electrical and electronic equipment.

ACKNOWLEDGMENTS

The valuable assistance and advice given by the following persons is gratefully acknowledged:

NRL: Messrs. Hayward Baker and Paul Leach for recommendations regarding the dewatering of electronic and electrical equipment.

NBL: Mr. John Schutz for still and moving picture photography and Mr. Fred Houth for experimental assistance.

NCEL: Dr. A. J. Paszyc and Messrs. N. P. Oldson, R. E. Jochums, and E. Beck for their interest and useful suggestions. Messrs. Oldson and G. W. Byrd were especially helpful in preparing all of the construction material samples.

NRDL: Messrs. Rolf Renner and Herbert Lacayo, Jr., for interest and assistance.

NBC Defense School: Lt. L. G. Beuchler for general arrangements and Messrs. Bowers and Skrove, the volunteer subjects in Experiments 1 and 2.

REFERENCES

1. Fielding, G.H., Neihof, R.A., and Echols, W.H., "Biological Warfare Decontamination with Hypochlorite," pp. 7-9 in Report of NRL Progress, Oct. 1968

2. Curran, H.R., "Resistance in Bacterial Spores," Part V, "Symposium on the Biology of Bacterial Spores," Bact. Reviews 16, 111-117 (1952)

3. Brazis, A., Leslie, E., Kabler, P.W., and Woodward, R.L., "Sporicidal Action of Free Available Chlorine," Special Report to Department of the Navy, Bureau of Yards and Docks by U.S. Department of Health, Education, and Welfare, Public Health Service, Robert A. Taft Sanitary Engineering Center, Apr. 1957

4. Brazis, A.R., Leslie, J.E., Kabler, P.W., and Woodward, R.L., "The Inactivation of Spores of *Bacillus globigii* and *Bacillus anthracis* by Free Available Chlorine," Appl. Microbiol. 6, 338-342 (1958)

5. Dychdala, G.R., "Chlorine and Chlorine Compounds," C.A. Lawrence and S.S. Block, editors, "Disinfection, Sterilization, and Preservation," Lea and Febiger, Phila., 1968, pp. 288-291

6. Charlton, D., and Levine, M., "Germicidal Properties of Chlorine Compounds," Iowa Engineering Experimentation Station Bulletin 132, Iowa State College, Ames, Iowa, 1932

7. Schwartz, A.M., Perry, J.W., and Berch, J., "Surface Active Agents and Detergents," Vol. II, Interscience, New York, 1958, p. 488

8. Marquis, R.E., and Gerhardt, P., "Polymerized Organic Salts of Sulfonic Acids Used as Dispersing Agents in Microbiology," J. Appl. Microbiology 7, 105-108 (1959)

9. Maybank, J., "The Physical Size Distribution of Bacterial Aerosols Produced by the Collison Sprayer," Suffield Technical Note 37, Suffield Experimental Station, Ralston, Alberta, July 1960

10. Miles, A.A., and Misra, S.S., "The Estimation of the Bactericidal Power of the Blood," J. Hyg. 28, 732-749 (1938)

11. Fielding, G.H., Echols, W.H., and Neihof, R.A., "BW Decontamination with Hypochlorite, Part 1 - Exploratory Studies: Application to the STOP System," NRL Report 6506, Feb. 28, 1967

12. "Chemical, Biological and Radiological (CBR) Decontamination," Department of the Army Technical Manual TM 3-220, Sept. 1961, later edition Nov. 1967

13. "ABC Defense Ashore," Department of the Navy, Bureau of Yards and Docks, Technical Publications NavDocks-TP-PL-2, revised Apr. 1960

U S GOVERNMENT PRINTING OFFICE 1970-397-177/227

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Naval Research Laboratory Washington, D.C. 20390	Unclassified
	2b GROUP

3 REPORT TITLE

DISINFECTION WITH HYPOCHLORITE: APPLICATION TO CLOTHED MEN, CONSTRUCTION MATERIALS, AND ELECTRONIC AND ELECTRICAL ITEMS

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

An interim report on a continuing NRL Problem.

5 AUTHOR(S) (First name, middle initial, last name)

G. H. Fielding, R. L. Dimmick, R. A. Neihof, and W. H. Echols

6 REPORT DATE	7a TOTAL NO OF PAGES	7b NO OF REFS
June 29, 1970	48	13

8a CONTRACT OR GRANT NO	9a ORIGINATOR'S REPORT NUMBER(S)
NRL Problem C08-22 b PROJECT NO S4801X2	NRL Report 7067
c d	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10 DISTRIBUTION STATEMENT

Each transmittal of this document outside the agencies of the U.S. Government must have prior approval of the Director, Naval Research Laboratory, Washington, D.C. 20390

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	Department of the Navy (Naval Ship Systems Command), Washington, D.C. 20360

13 ABSTRACT

The ASH/SLASH (Activated Solution of Hypochlorite/Self-Limiting Activated Solution of Hypochlorite) system, containing 0.2 percent hypochlorous acid, kills highly resistant bacterial spores on men and their clothing, on oily and greasy fabrics, on some two dozen different construction materials, and on some electrical and electronic items, without significant damage or degradation of the substrates.

DD FORM 1 NOV 65 1473 (PAGE 1)

S/N 0101-807-6801

Security Classification

Security Classification

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Disinfection						
Sprayers						
Hypochlorites						
Bactericides						
Microorganisms						
Bacillus anthracis						
Bacillus subtilis						
Spores						
Biological operations						
Clothing						
Humans						
Construction materials						
Electric equipment						
ASH						
SLASH						
Decontamination						

DD FORM 1 NOV 65 1473 (BACK)
(PAGE 2)

Security Classification